CHLORINATED PARAFFINS

INDUSTRY ASSOCIATION

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November 28, 2006

Document Control Office (DCO) (7407M)
Office of Pollution Prevention and Toxics (OPPT)
US Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001
ATTN: 8(d) Health and Safety Reporting Rule
(Notification/Reporting)

CONTAIN NO CBI

Re: TSCA 8(d) Regulations Promulgated on August 16, 2006 (71 FR 47130) and September 15, 2006 (71 FR 54434); Docket No. EPA-HQ-OPPT-2005-0055

Dear Document Control Officer:

On behalf of Dover Chemical Corporation, please accept this submission for **alkanes, chloro (CAS No. 61788-76-9)** in response to the final rule, "Health and Safety Data Reporting; Addition of Certain Chemicals," as published in the *Federal Register* on August 16, 2006 (71 FR 47130) and revised in the *Federal Register* on September 15, 2006 (71 FR 54434).

As noted in our August 30, 2006 submission, the chlorinated paraffins industry maintains that the submission of information on chlorinated paraffins, represented by alkanes, chloro (CAS No. 61788-76-9), should not be subject to this rule given that these compounds should not be considered "orphan" chemicals as all forms of chlorinated paraffins are covered by OECD and ICCA HPV programs.

Chlorinated paraffins are divided into three subcategories based on their carbon chain lengths:

- short-chain (carbon chain lengths 10-13);
- medium-chain (carbon chain lengths 14-17); and,
- long-chain (carbon chain lengths 18-30).

In our letter to USEPA of May 5, 1999, CPIA committed to address all forms of chlorinated paraffins represented on the US HPV Program candidate list including CAS Nos. 61788-76-9, 63449-39-8, 68920-70-7 and 68527-02-6. A follow-up communication was sent to EPA on February 28, 2002, notifying the Agency that the short and medium-chain chlorinated paraffins were being addressed by the OECD HPV program under the following names and CAS numbers: Alkanes, C_{10-13}, chloro (85535-84-8) and Alkanes,

300519

C_{14-17}, chloro (85535-85-9) and that the long-chain chlorinated paraffins would be covered under the ICCA HPV Initiative.

In response to our August 30 request to delist 61788-76-9, EPA disagreed with the premise that the OECD SIDS program sponsorship of short and medium-chain chlorinated paraffins was adequate to address the information needs for 61788-76-9. While we fail to understand how the Agency arrived at this position, we are nonetheless, submitting available health and safety studies. It is important to note that since the subject chemical – alkanes, chloro (CAS No. 61788-76-9) – can represent all chain length chlorinated paraffins, I have included in this submission unpublished studies on either short, medium or long-chain chlorinated paraffins. A list of the included studies is attached.

Please do not hesitate to contact me if I can be of any assistance at 202-419-1500 or by e-mail at rfensterheim@regnet.com.

Sincerely,

Robert J. Fensterheim

Robert J. Fensterheim
Executive Director

cc: Joe Nash (ccd.citb@epa.gov)

Prepared November 28, 2006

HEALTH AND SAFETY STUDIES FOR ALAKNES, CHLORO
(CAS No. 61788-76-9)

Unpublished Studies on Short, Medium and Long-Chain Chlorinated Paraffins

Elcombe, B.M. (2005). "A dietary study to determine the 90 day NOAEL of medium chain chlorinated paraffins (Cereclor S52) in male and female Fischer 344 rats." CXR Biosciences Ltd., CXR0273.

Elcombe, B.M. (2005). "Study to investigate the elimination of medium chain chlorinated paraffins in male F344 rats." CXR Biosciences Ltd., CXR0204.

Thompson, R.S. (2004). "Medium-chain chlorinated paraffin (C_{14-17}, 52% chlorinated): A comparison of acute toxicity to *Daphnia magna* using two different carrier solvents and water-accommodated fractions." AstraZeneca, BLS3192/B.

Thompson, R.S. (2005). "Long-chain chlorinated paraffin ($C_{>20}$, 43% chlorinated): Determination of acute toxicity to *Daphnia magna*." AstraZeneca, BLS3308/B.

List of Ongoing Health and Safety Studies

Study to assess the long-term biodegradation of short-chain chlorinated paraffins in sediment water systems simulating freshwater and esturine environments. The study is being conducted at AstraZeneca under the direction of Dr. Roy Thompson. This study is excepted to be completed in 2006.

Bioconcentration study of medium-chain chlorinated paraffins, according to OECD 305. This study was sponsored by the CEFIC Chlorianted Paraffins Sector Group and was conducted by AstraZeneca. The study is believed to be complete; an attempt is being made to obtain a copy of the study report.

CXR Biosciences Ltd.
James Lindsay Place
DUNDEE DD1 5JJ

DRAFT REPORT

A DIETARY STUDY TO DETERMINE THE 90 DAY NOAEL OF MEDIUM CHAIN CHLORINATED PARAFFINS (Cereclor S52) IN MALE AND FEMALE FISCHER 344 RATS.

CXR STUDY NUMBER:	CXR0273
Sponsor:	Eurochlor Av. E. van Nieuwehhuyse 4 Bt 2, B-1160 Brussels, Belgium.
Study Director:	B. M. Elcombe, CXR Biosciences
Principal Investigator (Pathology):	Dr Vasanthi Mowat, PHI Ltd.
Pathologist (Immunocytochemistry):	Dr Kevin Isaacs, CCRM Biotech Ltd.
Test facility:	CXR Biosciences, James Lindsay Place, Dundee Technopole, DundeeDD1 5JJ. and Ninewells Hospital and Medical School, Dundee, DD1 9SY; Medical School Resource Unit (MSRU), and Dept. of Clinical Pharmacology and Therapeutics.
Test site (Pathology):	PHI Ltd., Micron House, London Road, Harleston, Norfolk, IP20 9BH.
Test site (Immunocytochemistry):	CCRM Biotech Ltd. Unit 16, Cromarty Campus, Rosyth Europarc, Rosyth, Fife, KY11 2WX
"In Life" Start Dates:	23rd and 24th August 04
"In Life" Finish Dates:	22nd and 23rd November 04
CIRCULATION	Signed Original: Study File Sponsor Copies to: Study Director Sponsor

Author _____ _____
Barbara Elcombe, Study Director Date

Approved _____ _____
Director, CXR Biosciences Date

SUMMARY

1. Cereclor S52 was administered to male and female Fischer 344 rats in the diet for 90 days. Dietary concentrations of 30, 100, 300 and 3000ppm were utilised. The resultant systemic dose levels were: 2.38, 9.34, 23.0 and 222 mg/kg body weight per day for male rats and 2.51, 9.70, 24.6 and 242 mg/kg body weight per day for female rats. At this time the animals were sacrificed and parameters related to liver, thyroid and kidney structure and function examined.

2. Dietary administration of Cereclor S52 to rats for up to 90 days had no dose-related effect on terminal bodyweight or body weight gain.

3. In male rats both absolute and relative liver weights were significantly increased at the 3000ppm dose level to 113% and 117% of controls values respectively. Similarly, a 3000ppm total absolute and total relative kidney weights were increased to 109% and 112% of control values respectively. No effects on liver or kidney weights were observed at lower dose levels.

4. In female animals both absolute and relative liver weights were significantly increased at the 3000ppm dose level to 131% and 135% of control values respectively. No hepatic effects were seen at lower dose levels. At 3000ppm, total absolute and total relative kidney weights were increased to 113% and 116% of control values respectively.

5. Small decreases in plasma triglycerides and cholesterol were observed in male and female rats administered 3000ppm of Cereclor S52. These were the only significant effects of the test compound on any of the blood chemistry parameters measured.

6. Cereclor S52 at 300ppm and 3000ppm slightly decreased (to 74% and 78% of control values respectively) plasma free T3 in male rats. However, no effects were observed on plasma total T3 or free or total T4. A small increase in plasma TSH (117% of control) was noted in male rats at the 3000ppm dose level only. In female rats, Cereclor S52 had no effects on free or total plasma T3, or on total plasma T4. A small (20-39%), dose-related increase in plasma TSH was observed in the female animals at 300ppm and 3000ppm.

7. Cereclor S52 administration to male rats resulted in increased (172% of control values) hepatic microsomal T4-UDPGA transferase activity at 3000ppm. In female animals a dose-related effect was observed with increase above controls of 29%, 30% and 252%) respectively at 100, 300 and 3000ppm.

8. Administration of Cereclor S52 to male and female rats had no significant effects on

hepatic CN^- insensitive palmitoyl CoA oxidation.

9. Minimal centrilobular hepatocyte hypertrophy was noted in the livers of 9/10 male rats receiving 3000ppm Cereclor. This was not evident in male rats at the lower dose levels or in female rats at any dose. No treatment-related histopathology was reported in the kidneys or thyroids of male or female rats administered Cereclor S52 at dose levels of up to 3000ppm.

10. Cereclor S52 was without effect on α2u globulin protein expression levels in liver or kidney homogenates.

11. Based on these data we suggest a NOAEL of 300ppm for both male and female rats. In the current study, this equated to 23.0 and 24.6 mg/kg body weight for male and female rats respectively

CONTENTS

1. INTRODUCTION

Medium chain chlorinated paraffins (MCCPs) are chemical additives, used as plasticisers, fire retardants, in metal working fluids and the compounding of PVC.

This study was to ascertain a reliable No Adverse Effect Level (NOAEL) in male and female rats after a 90 day period of dietary exposure. These data will be used in human risk assessment.

This study used a rat *in vivo* model similar to that used in previous studies on MCCPs. The following parameters were studied:

- Thyroid, liver and kidney haematoxylin and eosin (H&E) histopathology on all samples.
- Clinical chemistry (16 parameters) on all samples.
- Evaluation of thyroid function – analysis of plasma levels of triiodothyronine (T3, Free and Total), thyroxine (T4, Free and Total) and thyroid stimulating hormone (TSH) on all samples.
- Acyl CoA oxidase activity was determined in liver heavy pellets as CN^--insensitive palmitoyl CoA oxidation. This was performed on all samples.
- T4-UDPGA glucuronosyl transferase activity in liver microsomes on all samples.
- Kidney α2u globulin immunocytochemistry on half the samples (males only).
- Quantification of α2u globulin by SDS-PAGE and Western blotting in liver and kidney homogenates on half the samples.

The Fischer 344 (F344) rat was the test system of choice for this study because previous work on MCCPs had been undertaken using this strain. In addition, CXR Biosciences has extensive experience in the use of this strain of rat, and the strain is well accepted by the regulatory authorities. The Sponsor requested the dietary route of administration.

2. LICENSEE RESPONSIBILITIES

The procedure performed was PD1 as detailed in the PD project licence (60/3005). The severity limit for this procedure is MODERATE.

3. MATERIALS AND METHODS

3.1 Test Item

The details outlined below are those provided by the Sponsor for the CXR Biosciences Test Item Data Sheet (TIDS). The Test Item was identified as Cereclor S52 unstabilised, and was supplied by INEOS Chlor Ltd. The Test Item was a clear pale yellow coloured liquid, supplied in unplasticised PE containers. The Test Item was despatched directly to the diet

preparation facility and stored at ~—20°C as specified in the TIDS.

3.2 Diet Preparation and Analysis

Preparation and analysis of the formulated diet was carried out at Inveresk Research, Tranent, EH33 3NE, Scotland, as a separate study, according to Inveresk Research protocol number 422393. A sample of Test Item and a sample of each concentration of each batch of diet formulated were retained (at ~—20°C, in glass or foil containers) by Inveresk Research.

Test Item, in sufficient quantity, was despatched to Inveresk Research by the Sponsor. The formulated diet was returned to CXR Biosciences, under ambient conditions, for presentation to the rats. Diets awaiting administration to the animals were stored at ~—20°C.

Analyses of the diet formulations were undertaken with regard to concentration, homogeneity and stability. Dose groups prepared at concentrations of 0ppm, 300ppm and 3000ppm were analysed by Inveresk Research, and the Certificate of Analysis provided by Inveresk Research is maintained in the Study file and as an appendix to the Study Report. Dose groups prepared at concentrations of 0ppm, 30ppm and 100ppm were analysed by CXR Biosciences and the Analytical Report is maintained in the Study file and as an appendix to the Study Report.

3.3 Safety Precautions

The normal safety precautions as detailed in the relevant SOPs and COSHH assessments applied, no additional precautions were considered necessary. Safety data sheets for the Test Item were supplied by the Sponsor.

3.4 Diet and Drinking Water

Control animals received powdered RM1 diet *ad libitum* for the duration of the study. The test groups of animals were administered Cereclor S52 in the diet *ad libitum* for the duration of the study. Diets awaiting administration to the animals were stored at ~—20°C.

Drinking water was taken from the local supply and provided, in bottles, *ad libitum.*

3.5 Animals

A sufficient number of male and female rats (5 – 8 weeks old on arrival) were obtained from Harlan UK Ltd (Shaw's Farm, Bicester, Oxfordshire, UK). On arrival in the Medical School Resources Unit the rats were randomly allocated to cages. They were housed on sawdust in solid-bottom, polypropylene cages. For this 90 day study no special arrangement of cages was used.

The rats were acclimatised for a period of at least 5 days before use.

3.6 Animal Accommodation and Husbandry

In the animal room the environment was controlled to provide conditions suitable for the F344 strain of rat. The temperature was maintained within a range of 19-23°C and relative humidity within a range of 40-70%. There were a nominal 14-15 air changes per hour. Twelve-hour periods of light were cycled with twelve-hour periods of darkness.

The rats were allowed water and powdered RM1 diet (supplied by Special Diet Services Ltd., Stepfield, Witham, Essex, UK) *ad libitum* until the start of the study.

4. EXPERIMENTAL DESIGN

The animals were uniquely numbered by ear-punch and allocated to groups up to one week after arrival. Group allocations were made in such a manner that the mean body weight in each group was similar. The rats were housed 2 per cage. An experimental card was placed on each cage and showed the project licence code, treatment group, study number, sex and individual numbers of the rats within, and identified the Home Office Licensee. In addition, these cards were colour coded to correlate with the coding for the treatment group.

The study consisted of one control group containing 20 male and 20 female animals, and four test groups, each containing 10 male and 10 female animals. Control animals received powdered RM1 diet *ad libitum* for the duration of the study. The test groups of animals were administered Cereclor S52 in the diet, *ad libitum*, for the duration of the study. The dietary concentrations of Cereclor S52 were 30, 100, 300, and 3000ppm of supplied chemical, without any correction for purity. All animals were sacrificed after 13 weeks (Table 1).

Table 1. **Experimental Design**

Group	Colour code	Rat No.	Sex	Dose (ppm)	Start Day	Kill Day
1	Blue	1 – 20	Male	0	Day1	Day 92
2	Green	21 – 30	Male	30	Day1	Day 92
3	Yellow	31 – 40	Male	100	Day1	Day 92
4	Red	41 – 50	Male	300	Day1	Day 92
5	White	51 – 60	Male	3000	Day1	Day 92
1	Blue	61 – 80	Female	0	Day2	Day 93
2	Green	81 – 90	Female	30	Day2	Day 93
3	Yellow	91 – 100	Female	100	Day2	Day 93
4	Red	101 – 110	Female	300	Day2	Day 93
5	White	111 – 120	Female	3000	Day2	Day 93

5. EXPERIMENTAL PROCEDURES

5.1 Bodyweight

The bodyweight of each rat was recorded at the start of the study. The animals were weighed weekly on the same day of the week. All animals were weighed prior to termination. The bodyweights were recorded in the Study Diary.

5.2 Clinical Observations

Prior to the start of the study, all rats were observed to ensure that they were physically normal and that they exhibited normal activity. Each rat was observed at least once daily during the study. There were no clinical abnormalities observed. The Study Diary was kept in the MSRU until completion of the in life phase of the study and then transferred to the Study File for retention and archiving.

5.3 Food Consumption

Food consumption was measured every time the diet jars were changed/refilled, and when the animals were sacrificed. In addition the diet jars were weighed on the same day of the week, regardless of refilling, so that weekly food consumption could be recorded.

5.4 Intercurrent Deaths

No rats required euthanasia during the study and there were no intercurrent deaths.

5.5 Terminal Procedures

On the day of termination the rats were weighed and transferred to a suitable room for post mortem. The rats were killed by exposure to a rising concentration of CO_2

Each carcass was processed as follows:

- Blood (approximately 1ml) was taken by cardiac puncture into tubes for serum.
- Blood (approximately 4.5ml, but as much as possible) was taken by cardiac puncture into lithium/heparin-coated tubes for plasma.
- The liver was removed from each animal and weighed.
    - 2 samples of liver, approximately 2mm strips, were taken, one from the Left lobe and one from the Median lobe. These were placed in neutral buffered formalin (NBF) for H&E histopathology provided by PHI Ltd.
    - Two chunks of liver (each approximately $1cm^3$) were flash frozen in liquid nitrogen and stored at ~-70°C for possible future analysis at the Sponsor's request.
    - One chunk of liver (weighing around 2 – 5g) was flash frozen in liquid nitrogen and stored at ~-70°C for future preparation of microsomes – see 5.6.3 below.
    - The remaining liver was weighed and used for the preparation of tissue homogenate and heavy pellet – see 5.6.3 below.
- The kidneys were removed from each animal and weighed individually.
    - A longitudinal section was taken from the middle of the left kidney, and a transverse section was taken from the middle of the right kidney. These were placed in NBF for H&E histopathology provided by PHI Ltd.
    - From the males only (rats 1 – 60) a further longitudinal section was taken from the middle of the left kidney, and a further transverse section was taken from the middle of the right kidney. These were placed in NBF for α2u immunocytochemistry provided by CCRM Biotech Ltd.
    - The remaining kidney tissue, for animals 1-10, 21-25, 31-35, 41-45, 51-55, 61-70, 81-85, 91-95, 101-105, 111-115, was weighed and used for the preparation of tissue homogenate – see 5.6.3 below.
    - The remaining kidney tissue, for animals 11-20, 26-30, 36-40, 46-50, 56-60, 71-80, 86-90, 96-100, 106-110 and 116-120 was placed in NBF and stored at room temperature for possible future analysis at the Sponsor's request.
- The thyroid (and attached parathyroid) was removed, together with the associated area of the trachea and part of the oesophagus. These were placed in NBF for H&E histopathology provided by PHI Ltd.

5.6 Sample Preparation

5.6.1 Serum / Plasma

Following removal into suitable tubes for serum or plasma preparation, venous blood samples were mixed on a roller for 10 min then cooled on ice. Clotted material, or red blood cells, were removed by centrifugation (3,000 rpm for 10 min at 8 - 10°C). The supernatant (serum or plasma) was stored at ~-20°C until required for analysis. The pellet was discarded.

5.6.2. Samples for histopathology

The samples of liver, kidney and thyroid from each rat were placed in NBF in screw topped plastic containers and allowed to fix at room temperature prior to shipping to PHI Ltd.

5.6.3 Tissue homogenates

Tissue homogenates were prepared from the remaining tissue of all the livers, and the kidneys of those animals specified above. Tissues were scissor-minced in ice-cold 1.15% (w/v) KCl to remove excess blood. The KCl was drained and a 25% (w/v) homogenate (liver) or 10% (w/v) homogenate (kidney) prepared in ice-cold SET buffer (0.25M Sucrose, 5mM EDTA and 20mM Tris-HCl, pH 7.4), (3ml SET/g liver, 9ml SET/g kidney). A Potter-Elvehjem homogeniser (6-8 passes) was used for liver samples, and a Polytron homogeniser was used for kidney samples. The volume of homogenate was not measured. Samples of liver and kidney homogenate were stored at ~-70°C pending analysis.

5.6.4 Liver heavy pellets

To prepare heavy pellet, the liver homogenate was centrifuged at 2,000rpm (Bench top Sorvall RT7 centrifuge, 10mins, ~4°C). The pellet was discarded and the resulting supernatant spun at 11,500rpm (Sorvall RC 28 S supraspeed centrifuge, 15mins, ~4°C). The pellet (heavy pellet) was resuspended in SET buffer (1g original liver/ml) and stored at ~-70°C pending analysis.

5.6.5 Liver microsomes

To prepare microsomes, the frozen liver chunks were thawed, weighed, and a 10% (w/v) homogenate prepared, as above. The liver homogenate was centrifuged at 2,000rpm (Bench top Sorvall RT7 centrifuge, 10mins, ~4°C). The pellet was discarded and the resulting supernatant spun at 11,500rpm (Sorvall RC 28 S supraspeed centrifuge, 15mins, ~4°C). The pellet (heavy pellet) was discarded and the resulting supernatant spun at 28,000rpm (Sorvall RC 28 S supraspeed centrifuge, 90mins, ~4°C). The pellets (microsomal fraction)

were resuspended in SET buffer (2g original liver/ml) and immediately used for the determination of T4-UDPGA glucuronosyl transferase activity. Any remaining microsomes were stored at ~-70°C for possible future analysis at the Sponsor's request. The supernatant (cytosol) was stored at ~-70°C for possible future analysis at the Sponsor's request.

5.7 Biochemical Measurements

5.7.1 Clinical chemistry

Serum samples, prepared as described above, were assayed for sodium and chloride using a Roche Integra 400 automated analyser according to the manufacturer's instructions. Any remaining serum was stored at ~-70°C for possible future analysis at the Sponsor's request.

Plasma samples, prepared as described above, were assayed for potassium, blood urea nitrogen (BUN), creatinine, total protein, albumin, globulin, ALT, AST, ALP, γGT, bilirubin, triglycerides, cholesterol and glucose. Any remaining plasma was stored at ~-70°C for possible future analysis at the Sponsor's request.

System control samples and calibration standards were those supplied by the manufacturer. Results are maintained in the Study File.

5.7.2 Thyroid function

Plasma samples, prepared as described above, were assayed for Free and Total T3, Free and Total T4, and TSH using a solid-phase ^{125}I radioimmunoassay. Results are maintained in the Study File.

T4-UDPGA glucuronosyl transferase activity was measured in liver microsomes, using ^{125}I labelled thyroxine and HPLC separation. Results are maintained in the Study File.

5.7.3 Vitamin K

Insufficient plasma samples remained for determination of vitamin K_1 (phylloquinone) content.

5.7.4 Protein determination

The protein concentration of tissue homogenates, heavy pellets and microsomes were determined using a modification of the method of Lowry *et al.*, (1951) and bovine serum albumin standards. Results are maintained in the Study File.

5.7.5 Peroxisome proliferation

CN^- insensitive acyl CoA oxidation was determined spectrophotometrically in liver heavy pellets, using palmitoyl CoA as a substrate. Results are expressed as nmol NAD^+ reduced/min/mg protein. Results are maintained in the Study File.

5.8 Histopathology

Following fixation at room temperature, thyroid, liver and kidney samples were transferred to PHI Ltd, Micron House, London Road, Harleston, Norfolk, IP20 9BH for processing and histopathological analysis (H&E). Samples were processed and 5 μM sections cut according to PHI Ltd SOPs. Sections were stained, using haematoxylin and eosin, again according to PHI Ltd SOPs. Samples were subjected to histopathological evaluation by Dr. Vasanthi Mowat, MRCVS, FRCPath. The histopathology report as provided by PHI Ltd will be maintained in the Study File and as an appendix to the Study Report.

Following fixation, the male kidney samples (1 – 60) were transferred to CCRM Biotech Ltd. Kidney α2u globulin immunocytochemistry was performed according to CCRM Biotech Ltd SOPs and the samples were evaluated by Dr. Kevin Isaacs. The immunocytochemistry report as provided by CCRM Biotech Ltd will be maintained in the Study File and as an appendix to the Study Report.

5.9 Western Blotting

Quantification of α2u globulin was carried out by SDS-PAGE/Western blotting of using liver and kidney homogenates and the subsequent image analysis of the immunoblots. This was carried out for the following samples only: 1-10, 21-25, 31-35, 41-45, 51-55, 61-70, 81-85, 91-95, 101-105, 111-115. Results are maintained in the Study File.

5.10 Additional Analyses

The Sponsor has requested no additional analyses. Samples of liver have been retained at ~-70°C. If further analysis is required these will be conducted under a separate Study. All other remaining samples will be discarded after the Final Report has been signed.

5.11 Protocol Amendments

No Protocol Amendments were issued for this Study.

5.12 Statistical Analysis

Statistical comparisons between treated and control groups have been undertaken for all

numerical data sets. The statistical analyses performed are documented with the results.

5.13 Data Storage and Archiving

The data will be held in a Study File according to CXR Biosciences SOPs 0003 and 0005. On completion of the study, the following items will be retained in the CXR Archive:

- Study protocol and possible amendments
- Study Report and possible amendments
- Study File (including Study Diary)
- Relevant correspondence

Histopathology specimens will be retained by PHI Ltd for a period of two years.

All items will be kept for two years from the date of signing the Study Report unless, in the opinion of CXR Biosciences or PHI Ltd, the quality of the specimens no longer allows evaluation. At the end of the two year period, the Study Sponsor will be contacted and the items either sent to the Study Sponsor or retained by CXR Biosciences/ PHI Ltd. under a separate agreement.

6. RESULTS

6.1 Diet Analysis

Diets, stored at ~–20°C, were analysed for achieved concentrations of Cereclor S52 by CXR Biosciences (30 and 100ppm) and Inveresk Research (300 and 3000ppm) (Table 2).

Table 2. Achieved Concentrations of Cereclor S52 in Experimental Diets

		Achieved Concentrations (ppm)					
Preparation date	Target ppm	Mean ppm	Coefficient of variation (%)	% Difference from target ppm	Overall mean ppm	Overall coefficient of variation (%)	Overall % difference from target ppm
12/08/04	0	0	-	-	0	-	-
10/09/04	0	0	-	-			
07/10/04	0	0	-	-			
12/08/04	30	37	0.0	23	31	16.9	3
10/09/04	30	26	15.3	-12			
07/10/04	30	29	5.2	-2			
12/08/04	100	126	2.1	26	123	5.6	23
10/09/04	100	119	8.4	19			
07/10/04	100	123	4.9	23			
12/08/04	300	310	2.1	3	300	4.0	0
10/09/04	300	300	3.9	0			
07/10/04	300	290	3.0	-3			
12/08/04	3000	2924	2.6	-3	2981	3.5	-1
10/09/04	3000	3052	4.4	2			
07/10/04	3000	2967	1.8	-1			

6.2 Bodyweights

Dietary administration of Cereclor S52 to rats for up to 90 days had no dose-related effect on terminal bodyweight (Bwt) or body weight gain (Figures 1 and 2; Tables 3 and 4).

Figure 1. Effect of Cereclor S52 on Male Rat Bodyweights with Time

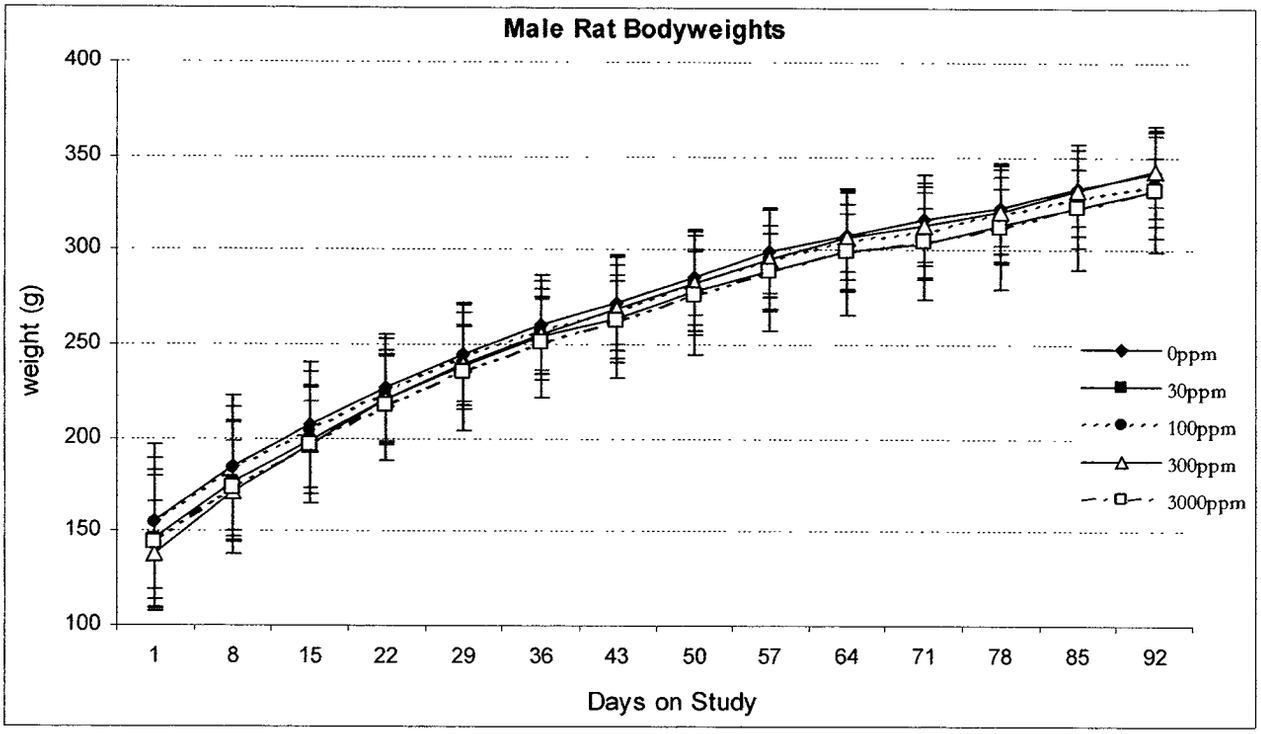


Table 3. Effect of Cereclor S52 on Male Rat Bodyweights with Time

Day	Bodyweight (g)				
	0ppm	30ppm	100ppm	300ppm	3000ppm
1	155±41	146±36	154±35	138±29	144±36
8	185±38	177±32	183±33	171±27	173±35
15	207±34	199±29	204±31	196±23	196±31
22	227±29	220±24	225±28	221±22	217±29
29	245±27	239±21	244±28	240±21	235±31
36	260±26	255±20	257±26	255±19	251±29
43	272±25	263±21	268±28	268±18	262±30
50	285±25	278±21	282±27	283±17	276±31
57	299±24	289±20	294±26	296±18	289±32
64	308±23	299±20	304±27	307±18	299±33
71	316±24	304±19	310±25	313±19	305±31
78	322±24	313±20	320±25	321±18	311±32
85	332±25	322±21	327±26	332±18	322±32
92	342±24	331±19	335±28	342±19	332±32

Diet administered on Day 1. Values are Mean ± SD

n = 20 per group for the control group, and 10 per group for all test groups.

A Student's t-test (2-sided) was performed on the results; * = statistically different from control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

Figure 2. Effect of Cereclor S52 on Female Rat Bodyweights with Time

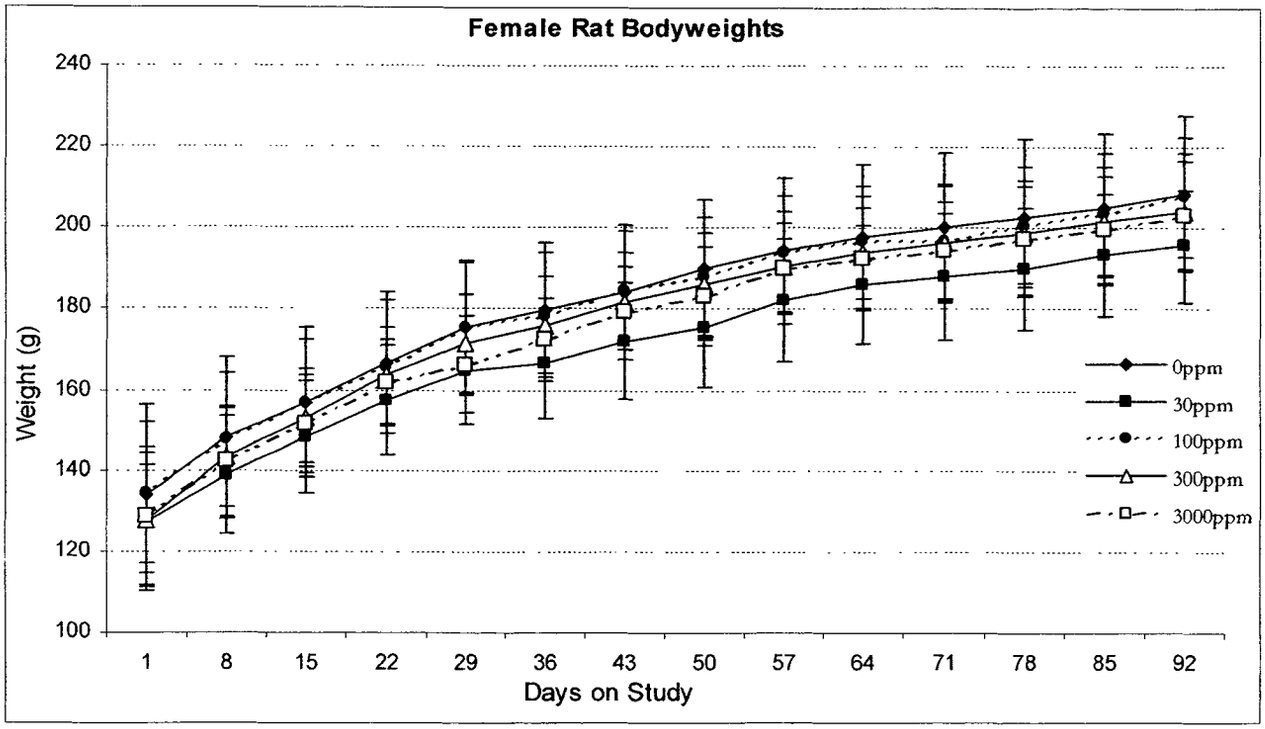


Table 4. Effect of Cereclor S52 on Female Rat Bodyweights with Time

Day	Bodyweight (g)				
	0ppm	30ppm	100ppm	300ppm	3000ppm
2	134±23	127±17	135±18	128±13	129±17
9	148±20	139±15	148±16	143±12	142±14
16	157±18	148±14	157±15	153±12	151±12
23	167±17	158±14	166±17	163±12	162±11
30	175±17	165±13	175±16	172±12	166±12
37	180±17	167±14*	178±15	176±12	172±10
44	184±17	172±14*	185±15	182±12	179±12
51	190±17	175±15*	188±15	186±13	183±12
58	194±18	182±15	194±14	190±14	190±11
65	198±18	186±15	197±14	194±14	192±12
72	200±18	188±15	197±14	196±14	194±12
79	203±19	190±15	201±14	199±13	197±13
86	205±19	193±15	204±15	202±14	200±13
93	209±19	196±14*	208±15	204±15	203±13

Diet administered on Day 2. Values are Mean ± SD

n = 20 per group for the control group, and 10 per group for all test groups.

A Student's t-test (2-sided) was performed on the results; * = statistically different from control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

6.3 Food Consumption

No significant effects on food consumption were observed over the experimental period (Tables 5 and 6).

Table 5. Effect of Cereclor S52 on Male Rat Food Consumption with Time

Day	Diet Consumed (g) / Rat / Week				
	0ppm	30ppm	100ppm	300ppm	3000ppm
8	129±8	130±8	129±5	132±6	126±10
15	133±6	133±5	133±6	135±5	128±8
22	131±7	135±8	134±6	137±7	129±9
29	142±12	142±11	144±6	145±9	135±12
36	138±11	142±9	141±5	141±9	132±12
43	136±10	139±10	144±7	141±8	133±13
50	140±12	138±13	145±7	141±9	136±13
57	137±11	136±11	143±7	140±9	136±11
64	143±11	146±14	148±11	145±9	138±10
71	145±15	148±11	148±11	145±9	141±12
78	145±12	150±14	149±7	140±8	141±13
85	139±8	145±16	140±10	155±5	135±10
92	135±8	137±13	139±12	137±7	135±10

Diet administered on Day 1. Values are Mean ± SD

n = 20 per group for the control group, and 10 per group for all test groups.

A Student's t-test (2-sided) was performed on the results; * = statistically different from control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

Table 6. Effect of Cereclor S52 on Female Rat Food Consumption with Time

Day	Diet Consumed (g) / Rat / Week				
	0ppm	30ppm	100ppm	300ppm	3000ppm
9	99±6	94±4	100±7	99±6	95±3
16	99±5	99±4	100±5	101±8	97±3
23	101±5	99±4	101±6	104±7	99±4
30	103±5	101±5	103±5	106±4	99±5
37	103±6	97±5	102±3	104±4	107±11
44	99±7	95±5	99±5	97±10	101±5
51	102±6	94±7	100±4	101±3	101±5
58	102±6	100±3	103±6	102±3	104±4
65	105±6	102±5	106±6	108±3	107±5
72	104±4	101±6	101±7	107±2	104±5
79	102±4	97±5	102±6	107±3	103±5
86	99±6	95±5	97±5	103±2	100±3
93	95±6	95±5	97±5	101±4	100±4

Diet administered on Day 2. Values are Mean ± SD

n = 20 per group for the control group, and 10 per group for all test groups.

A Student's t-test (2-sided) was performed on the results; * = statistically different from control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

6.4 Ingested Dose of Cereclor S52 by Male and Female Rats

The ingested doses of Cereclor S52 are shown in Table 7.

Table 7. Ingested Dose of Cereclor S52 by Male and Female Rats

Group	Sex	Dose (ppm)	Actual Dose (ppm)	Diet ingested (g diet/kg Bwt/day)	MCCP ingested (mg/kg Bwt/day)
1	Male	0	0	73.3±13.0	0.00±0.00
2	Male	30	31	76.9±13.5	2.38±0.03
3	Male	100	123	75.9±12.3	9.34±0.11
4	Male	300	300	76.7±15.3	23.01±0.35
5	Male	3000	2981	74.6±14.0	222.46±3.11
1	Female	0	0	78.9±8.8	0.00±0.00
2	Female	30	31	81.1±9.0	2.51±0.02
3	Female	100	123	78.9±8.8	9.70±0.09
4	Female	300	300	82.1±8.6	24.62±0.21
5	Female	3000	2981	81.5±8.0	242.83±1.93

6.5 Terminal Organ and Bodyweights

In male rats both absolute and relative liver weights (organ/bodyweight ratio) were significantly increased at the 3000ppm dose level to 113% and 117% of controls values respectively (Table 8). Similarly, at 3000ppm total absolute and total relative kidney weights were increased to 109% and 112% of control values respectively (Table 8). No effects on liver or kidney weights were observed at lower dose levels.

Larger effects were seen in the liver of female animals, where both absolute and relative liver weights were significantly increased at the 3000ppm dose level to 131% and 135% of controls values respectively (Table 9). No hepatic effects were seen at lower dose levels. At 3000ppm total absolute and total relative kidney weights were increased to 113% and 116% of control values respectively (Table 9). A small 7% increase in relative kidney weight only was observed at 300ppm.

Table 8. Effect of Cereclor S52 on Male Rat Organ and Bodyweights (g)

Parameter	0ppm	30ppm	100ppm	300ppm	3000ppm
Bodyweight (Bwt)	342±24 (100±7)	331±19 (97±5)	335±28 (98±8)	342±19 (100±5)	332±32 (97±9)
Liver weight	11.614±1.455 (100±13)	11.374±1.021 (98±9)	11.406±1.194 (98±10)	11.927±1.099 (103±9)	13.152±1.422 (113±12) **
Liver/Bwt Ratio %	3.396±0.307 (100±9)	3.431±0.200 (101±6)	3.408±0.222 (100±7)	3.482±0.170 (103±5)	3.967±0.219 (117±6) ***
L. Kidney weight	0.959±0.104 (100±11)	0.935±0.085 (97±9)	0.952±0.077 (99±8)	0.986±0.085 (103±9)	1.044±0.107 (109±11) *
R. Kidney weight	0.954±0.108 (100±11)	0.946±0.083 (99±9)	0.938±0.088 (98±9)	0.984±0.064 (103±7)	1.033±0.096 (108±10)
Total Kidney wt.	1.913±0.207 (100±11)	1.881±0.162 (98±8)	1.890±0.162 (99±8)	1.970±0.145 (103±8)	2.076±0.201 (109±11) *
Kidney/Bwt Ratio %	0.559±0.035 (100±6)	0.567±0.027 (101±5)	0.565±0.029 (101±5)	0.576±0.026 (103±5)	0.627±0.028 (112±5) ***

Values are Mean ± SD (% control)

n = 20 per group for the control group, and 10 per group for all test groups.

A Student's t-test (2-sided) was performed on the results; * = statistically different from control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

Table 9. Effect of Cereclor S52 on Female Rat Organ and Bodyweights (g)

Parameter	0ppm	30ppm	100ppm	300ppm	3000ppm
Bodyweight (Bwt)	209±19 (100±9)	196±14 (94±7) *	208±15 (100±7)	204±15 (98±7)	203±13 (97±6)
Liver weight	5.978±0.762 (100±13)	5.869±0.555 (98±9)	6.091±0.590 (102±10)	6.153±0.529 (103±9)	7.854±0.508 (131±9) ***
Liver/Bwt Ratio %	2.868±0.250 (100±9)	3.003±0.238 (105±8)	2.937±0.262 (102±9)	3.014±0.154 (105±5)	3.862±0.173 (135±6) ***
L. Kidney weight	0.608±0.054 (100±9)	0.597±0.064 (98±11)	0.629±0.046 (103±8)	0.634±0.055 (104±9)	0.686±0.044 (113±7) ***
R. Kidney weight	0.609±0.057 (100±9)	0.599±0.055 (98±9)	0.631±0.052 (104±9)	0.637±0.058 (105±9)	0.693±0.041 (114±7) ***
Total Kidney wt.	1.217±0.108 (100±9)	1.197±0.116 (98±10)	1.260±0.097 (104±8)	1.271±0.111 (104±9)	1.380±0.078 (113±6) ***
Kidney/Bwt Ratio %	0.584±0.025 (100±4)	0.612±0.045 (105±8)	0.607±0.036 (104±6)	0.622±0.037 (107±6) **	0.679±0.026 (116±4) ***

Values are Mean ± SD (% control)

n = 20 per group for the control group, and 10 per group for all test groups.

A Student's t-test (2-sided) was performed on the results; * = statistically different from control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

6.6 Biochemical Measurements

6.6.1 Clinical chemistry

Small decreases in triglycerides and cholesterol were observed in male and female rats administered 3000ppm of Cereclor S52. These were the only significant effects of the test compound on any of the blood chemistry parameters measured (Tables 10 and 11). Measurements of conjugated bilirubin were very near the lower limits of detection of the assay.

For all clinical chemistry parameters shown below, values are expressed as Mean ± SD (% control). n =20 for the control groups and 10 for all of the test groups.

Total protein and globulin, n = 20 per group for the male control group, 9 per group for the male 100ppm group, and 10 per group for the remaining male test groups.

Sodium, n = 16 per group for the female control group, and 10 per group for the remaining female test groups.

A Student's t-test (2-sided) was performed on the results; with * statistically different from control at $p<0.05$; ** statistically different from control at $p<0.01$; and *** statistically different from control at $p<0.001$.

Table 10. Effect of Cereclor S52 on Male Rat Clinical Chemistry Parameters

Parameter	0ppm	30ppm	100ppm	300ppm	3000ppm
Albumin g/L	32.4±2.2 (100±7)	33.3±1.9 (103±6)	31.8±2.0 (98±6)	31.2±2.7 (96±8)	30.2±3.3 (93±10)
ALP U/L	134.5±17.7 (100±13)	149.9±20.9 (111±16)	133.3±11.8 (99±9)	132.8±17.2 (99±13)	126.4±22.7 (94±17)
ALT U/L	70.5±9.1 (100±13)	91.4±20.3 (130±29) **	79.0±20.7 (112±29)	67.7±16.9 (96±24)	70.3±13.4 (100±19)
AST U/L	74.7±11.7 (100±16)	86.2±17.8 (115±24)	76.2±18.8 (102±25)	81.1±35.8 (109±48)	68.4±10.0 (92±13)
Total Bilirubin (BIL) µmol/L	13.7±5.7 (100±42)	12.7±4.8 (93±35)	16.9±7.7 (124±57)	13.0±2.7 (95±19)	12.5±4.4 (91±32)
Conjugated BIL µmol/L	0.117±0.163 (100±140)	0.107±0.103 (92±89)	0.142±0.178 (122±153)	0.118±0.137 (101±118)	0.022±0.047 (19±41) *
Unconjugated BIL µmol/L	13.6±5.8 (100±42)	12.6±4.9 (93±36)	16.8±7.8 (124±57)	12.8±2.6 (95±20)	12.5±4.4 (92±33)
Cholesterol mmol/L	1.661±0.286 (100±17)	1.515±0.150 (91±9)	1.622±0.196 (98±12)	1.609±0.181 (97±11)	1.275±0.152 (77±9)
Creatinine µmol/L	49.5±3.2 (100±7)	49.3±4.0 (100±8)	49.8±2.7 (101±6)	47.1±3.9 (95±8)	49.5±5.7 (100±12)
gGT U/L	Below limit of detection	Below limit of detection	Below limit of detection	Below limit of detection	Below limit of detection
Globulin g/L	28.8±4.9 (100±17)	31.0±4.0 (108±14)	25.9±2.2 (90±8)	29.2±9.2 (101±32)	30.2±8.4 (105±29)
Glucose mmol/L	13.6±2.2 (100±17)	14.6±1.8 (108±13)	15.8±1.5 (116±11)	13.5±2.0 (99±15)	12.4±2.0 (91±14)
Potassium mmol/L	5.93±0.70 (100±12)	6.85±1.20 (115±20)	6.76±1.55 (114±26)	5.75±1.10 (97±18)	5.13±0.76 (87±13)
Total Protein g/L	61.2±4.9 (100±8)	64.3±4.8 (105±8)	57.8±3.0 (94±5) *	60.3±9.4 (99±15)	60.4±10 (99±17)
Triglycerides mmol/L:	1.171±0.456 (100±39)	1.172±0.375 (100±32)	1.487±0.612 (127±52)	1.225±0.155 (105±13)	0.844±0.355 (72±30) *
Urea mmol/L	4.98±0.87 (100±17)	4.93±0.55 (99±11)	5.39±0.60 (108±12)	4.88±0.59 (98±12)	4.95±0.52 (99±10)
Sodium+ mmol/L	157.0±6.4 (100±4)	152.8±2.5 (97±2)	158.0±10 (101±6)	154.6±6.4 (98±4)	160.7±11.8 (102±7)
Chloride- mmol/L	101.5±2.8 (100±3)	98.4±2.9 (97±3) **	100.8±4.6 (99±5)	99.8±4.4 (98±4)	103.5±5.7 (102±6)

Table 11. Effect of Cereclor S52 on Female Rat Clinical Chemistry Parameters

Parameter	0ppm	30ppm	100ppm	300ppm	3000ppm
Albumin g/L	34.1±2.4 (100±7)	35.7±2.0 (105±6)	35.8±2.2 (105±6)	36.5±1.6 (107±5) **	36.2±3.3 (106±10)
ALP U/L	113.0±22.9 (100±20)	110.6±21.0 (98±19)	108.6±37.3 (96±33)	112.6±20.3 (100±18)	96.7±10.8 (86±10) *
ALT U/L	67.8±13.4 (100±20)	65.8±14.7 (97±22)	66.2±16.0 (98±24)	69.7±11.7 (103±17)	67.5±20.6 (100±30)
AST U/L	96.6±20.2 (100±21)	94.9±12.3 (98±13)	94.8±26.2 (98±27)	95.6±19.8 (99±20)	96.7±31.5 (100±33)
Total Bilirubin (BIL) µmol/L	11.1±5.0 (100±45)	12.1±5.0 (109±45)	9.7±3.0 (87±27)	7.1±1.7 (64±15) **	8.3±6.8 (75±61)
Conjugated BIL µmol/L	0.261±0.264 (100±101)	0.260±0.227 (100±87)	0.188±0.157 (72±60)	0.150±0.137 (58±53)	0.030±0.043 (12±16) ***
Unconjugated BIL µmol/L	10.8±5.0 (100±46)	11.8±5.0 (109±47)	9.5±3.1 (87±28)	6.9±1.7 (64±16) **	8.3±6.8 (77±62)
Cholesterol mmol/L	2.389±0.288 (100±12)	2.383±0.225 (100±9)	2.476±0.339 (104±14)	2.534±0.226 (106±9)	2.063±0.187 (86±8) ***
Creatinine µmol/L	51.5±5.0 (100±10)	53.3±4.0 (104±8)	53.3±5.4 (104±10)	53.9±7.3 (105±14)	49.1±5.1 (95±10)
gGT U/L	Below limit of detection	Below limit of detection	Below limit of detection	Below limit of detection	Below limit of detection
Globulin g/L	28.2±4.8 (100±17)	32.4±18.2 (115±64)	31.3±13.0 (111±46)	29.3±2.4 (104±8)	27.3±1.9 (97±7)
Glucose mmol/L	12.3±3.6 (100±29)	16.5±2.1 (134±17)***	16.3±2.3 (132±18)***	13.8±3.3 (112±27)	10.3±4.1 (84±34)
Potassium mmol/L	5.79±0.91 (100±16)	6.05±0.48 (104±8)	6.18±1.22 (107±21)	5.73±0.39 (99±7)	6.09±0.71 (105±12)
Total Protein g/L	62.3±4.6 (100±7)	68.1±18.4 (109±29)	67.1±12.3 (108±20)	65.8±3.3 (106±5) *	63.5±5.0 (102±8)
Triglycerides mmol/L:	0.644±0.241 (100±37)	0.838±0.315 (130±49)	0.773±0.172 (120±27)	0.637±0.140 (99±22)	0.393±0.057 (61±9) ***
Urea mmol/L	5.55±1.06 (100±19)	5.53±0.39 (100±7)	5.83±0.83 (105±15)	5.84±0.60 (105±11)	5.83±0.86 (105±16)
Sodium mmol/L	160.8±5.8 (100±4)	166.0±6.8 (103±4)	161.4±5.9 (100±4)	160.9±4.9 (100±3)	158.5±4.4 (99±3)
Chloride mmol/L	104.0±5.0 (100±5)	106.0±3.3 (102±3)	104.1±4.1 (100±4)	104.1±2.4 (100±2)	104.5±2.5 (101±2)

6.6.2 Thyroid function

6.6.2.1 Plasma total T3, free T3, total T4, free T4 and TSH

Cereclor S52 at 300ppm and 3000ppm slightly decreased (to 74% and 78% of control values respectively) plasma free T3 in male rats. However, no effects were observed on plasma total T3 or free or total T4. A small increase in plasma TSH (117% of control) was noted in male rats at the 3000ppm dose only (Table 12).

In female rats, Cereclor S52 had no effects on free or total plasma T3, or on total plasma T4. A statistically significant increase (141% of control values) in plasma free T4 was observed but the biological significance of this is obscure and is thought unlikely to be of toxicological significance. A small (20-39%), dose-related increase in plasma TSH was observed in the female animals at 300ppm and 3000ppm (Table 13).

Table 12. Effect of Cereclor S52 on Male Rat Thyroid Function Parameters

Parameter	0ppm	30ppm	100ppm	300ppm	3000ppm
Total T3 nmol/L	1.70±0.28 (100±16)	1.85±0.32 (109±19)	1.95±0.47 (114±28)	1.63±0.28 (96±17)	1.62±0.33 (95±19)
Free T3 pmol/L	2.90±1.12 (100±39)	2.42±0.54 (83±19)	2.41±0.28 (83±10)	2.15±0.34 (74±12) *	2.26±0.49 (78±17) *
Total T4 nmol/L	59.24±7.42 (100±13)	57.88±8.12 (98±14)	66.00±10.81 (111±18)	57.31±6.76 (97±11)	55.32±5.72 (93±10)
Free T4 pmol/L	25.18±2.42 (100±10)	24.76±3.62 (98±14)	26.89±3.59 (107±14)	26.44±2.64 (105±10)	24.08±2.41 (96±10)
TSH µg/L	7.46±0.93 (100±13)	8.44±1.59 (113±21)	7.46±1.71 (100±23)	7.07±1.34 (95±18)	8.74±0.86 (117±12)***

Values are Mean ± SD (% control)

n = 20 per group for the control group, and 10 per group for all test groups.

Free T3, n = 19 per group for the control group, and 10 per group for all test groups.

A Student's t-test (2-sided) was performed on the results; * = statistically different from control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

Table 13. Effect of Cereclor S52 on Female Rat Thyroid Function Parameters

Parameter	0ppm	30ppm	100ppm	300ppm	3000ppm
Total T3 nmol/L	2.70±0.66 (100±25)	2.64±1.07 (98±40)	2.54±0.78 (94±29)	2.44±0.45 (90±17)	2.25±0.51 (83±19)
Free T3 pmol/L	2.24±0.48 (100±21)	2.17±0.48 (97±22)	2.31±0.47 (103±21)	2.43±0.79 (108±35)	2.51±0.92 (112±41)
Total T4 nmol/L	46.68±11.84 (100±25)	41.45±9.30 (89±20)	44.44±7.32 (95±16)	52.90±11.88 (113±25)	55.20±17.73 (118±38)
Free T4 pmol/L	17.30±4.94 (100±29)	15.69±5.53 (91±32)	14.34±4.74 (83±27)	19.84±7.74 (115±45)	24.39±7.48 (141±43) *
TSH µg/L	6.08±0.88 (100±14)	6.52±1.52 (107±25)	7.38±2.40 (121±39)	7.31±1.09 (120±18) **	8.44±1.14 (139±19)***

Values are Mean ± SD (% control)

n = 20 per group for the control group, and 10 per group for all test groups.

Total T3, n = 19 per group for the control group, 8 per group for the 30ppm group, and 10 per group for the remaining test groups.

Free T3, n = 18 per group for the control group, 8 per group for the 30ppm group, 9 per group for the 100ppm group, and 10 per group for the remaining test groups.

A Student's t-test (2-sided) was performed on the results; * = statistically different from control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

6.6.2.2 Microsomal T4-UDPGA Transferase Activity

Cereclor S52 administration to male rats resulted in increased (172% of control values) hepatic microsomal T4-UDPGA transferase activity at 3000ppm. In female animals a dose-related effect was observed with increase above controls of 29%, 30% and 252%) respectively at 100, 300 and 3000ppm (Table 14).

Table 14. Effect of Cereclor S52 on Liver Microsomal T4-UDPGA Activity, expressed as T4 Glucuronides produced, pmol/minute/mg protein.

Sex	0ppm	30ppm	100ppm	300ppm	3000ppm
Male	0.612±0.190 (100±31)	0.488±0.114 (80±19) *	0.488±0.102 (80±17) *	0.555±0.172 (91±28)	1.113±0.310 (182±51)***
Female	0.404±0.085 (100±21)	0.440±0.057 (109±14)	0.525±0.087 (130±22) **	0.527±0.148 (130±37) *	1.429±0.375 (354±93)***

Values are Mean ± SD (% control)

n = 20 per group for the control group, and 10 per group for all test groups.

A Student's t-test (2-sided) was performed on the results; * = statistically different from

control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

6.6.3 Peroxisome proliferation

Administration of Cereclor S52 to male and female rats had no significant effects on hepatic CN^- insensitive palmitoyl CoA oxidation (Table 15).

Table 15. Effect of Cereclor S52 on Liver Heavy Pellet CN^- insensitive acyl CoA Oxidation, expressed as nmol NAD^+ reduced/minute/mg protein.

Sex	0ppm	30ppm	100ppm	300ppm	3000ppm
Male	11.86±2.39 (100±20)	10.96±2.50 (92±21)	9.50±2.63 (80±22) *	11.32±2.29 (95±19)	13.05±3.69 (110±31)
Female	12.73±1.99 (100±16)	12.52±1.22 (98±10)	11.81±2.73 (93±21)	11.87±1.55 (93±12)	13.43±1.65 (106±13)

Values are Mean ± SD (% control)
n = 20 per group for the control group, and 10 per group for all test groups.
A Student's t-test (2-sided) was performed on the results; * = statistically different from control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

6.7 Histopathology

6.7.1 Haematoxylin and Eosin Staining

Minimal centrilobular (periacinar) hepatocyte hypertrophy was noted in the livers of 9/10 male rats receiving 3000ppm Cereclor. This was not evident in male rats at the lower dose levels or in female rats at any dose (Table 16 and 17).

No treatment-related histopathology was reported in the kidneys or thyroids of male or female rats administered Cereclor S52 at dose levels of up to 3000ppm (Appendix 8 for report and data).

Table 16. Summary of Histopathology Data - Male Fischer 344 Rats.

	Concentration Cereclor S52 in Diet (ppm)				
	0	30	100	300	3000
Number of animals examined	20	10	10	10	10
Kidneys examined x 2					
Basophilic tubules, total	20	10	10	10	10
minimal	9	7	3	3	0
mild	11	3	7	7	10
Lymphocytic infiltration, total	4	3	0	3	0
minimal	4	3	0	3	0
Protein casts, total	2	1	2	1	3
minimal	2	1	2	1	3
Cortical tubular eosinophilic droplets, total	1	0	0	2	3
minimal	1	0	0	2	3
Interstitial nephritis, total	0	1	0	0	0
minimal	0	1	0	0	0
Livers examined x 2	20	10	10	10	10
Periacinar hepatocyte hypertrophy, total	0	0	0	0	9
minimal	0	0	0	0	9
Inflammatory cell foci, total	16	8	8	9	9
minimal	16	8	8	9	9
Periacinar cell vacuolation, total	1	0	0	0	0
minimal	1	0	0	0	0
Thyroids examined	19	10	10	10	10
Ultimobranchial cyst	1	1	0	3	1
Ectopic thymus	0	0	0	0	1
Follicular cell adenoma	0	0	0	0	1
Lymphocyte infiltration, total	0	1	0	0	0
minimal	0	1	0	0	0

Table 17. Summary of Histopathology Data - Female Fischer 344 Rats.

	Concentration Cereclor S52 in Diet (ppm)				
	0	30	100	300	3000
Number of animals examined	20	10	10	10	10
Kidneys examined x2	20	10	10	10	10
Basophilic tubules, total	3	4	3	6	5
minimal	3	4	3	6	5
Corticomedullary nephrocalcinosis, total	10	4	4	4	2
minimal	10	4	4	4	2
Medullary cyst, total	0	0	0	1	0
mild	0	0	0	1	0
Lymphocytic infiltration, total	5	0	0	1	1
minimal	5	0	0	1	1
Protein casts, total	1	2	0	0	1
minimal	1	2	0	0	1
Livers examined x2	20	10	10	10	10
Inflammatory cell foci, total	18	8	10	9	9
minimal	18	8	10	9	9
Pale cell foci, total	0	0	0	1	0
minimal	0	0	0	1	0
Thyroids examined	19	10	10	10	10
Ultimobranchial cyst	1	2	0	1	0
C-cell hyperplasia, total	0	1	1	0	0
minimal	0	1	1	0	0

6.7.2 Immunocytochemistry

There was no discernible pattern of specific staining for α2u globulin in any of the kidney sections examined. The lack of low-level detection of α2u globulin in the control male animals suggests that the immunohistochemical staining did not work (Appendix 9).

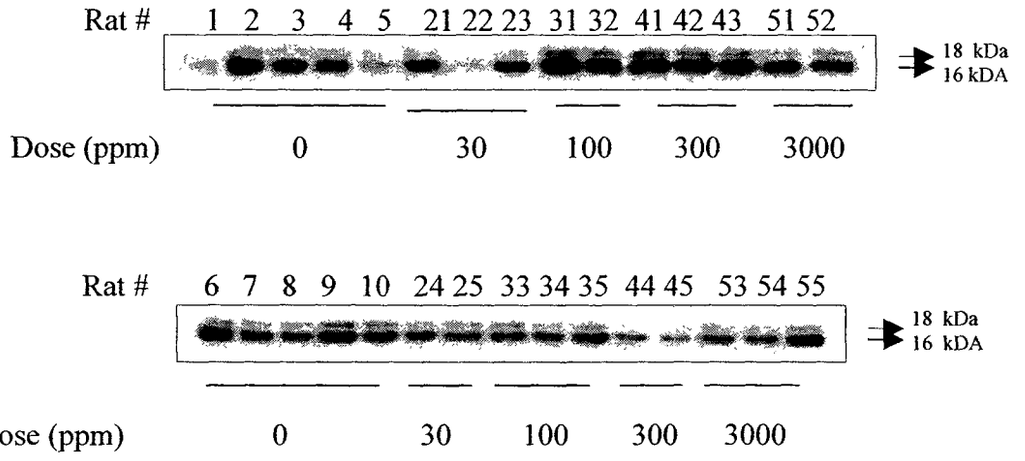
6.8 Western Blotting

SDS-PAGE followed by western blotting for α2u globulin revealed the expected presence of two isoforms (18 and 16 kD) in homogenates of male rat kidneys (Figure 3). Neither of these isoforms was increased by Cereclor S52 (Figure 3 and Table 18). As expected α2u globulin was not detected in female rat kidney homogenates (Figure 3).

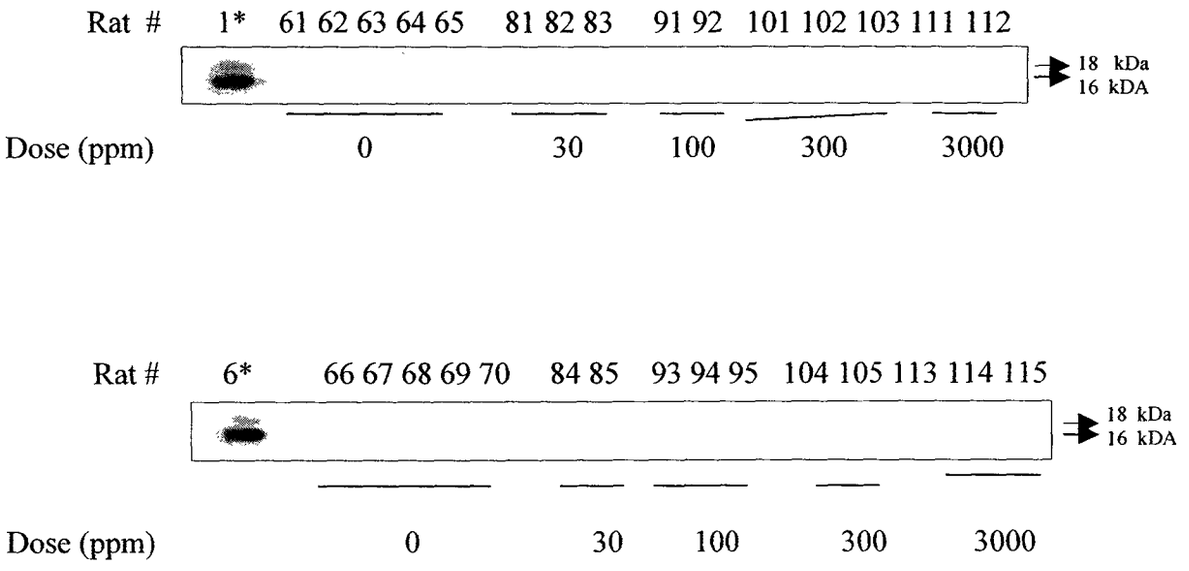
SDS-PAGE followed by western blotting for α2u globulin revealed the expected presence of one isoform in homogenates of male rat liver (Figure 4). The expression of α2u globulin was unaffected by Cereclor S52 administration (Figure 4 and Table 18). As expected α2u globulin was not detected in female rat liver homogenates (Figure 4).

Figure 3. α2u Globulin Expression in Kidneys of Male and Female Rats # 1-115

Male Kidney



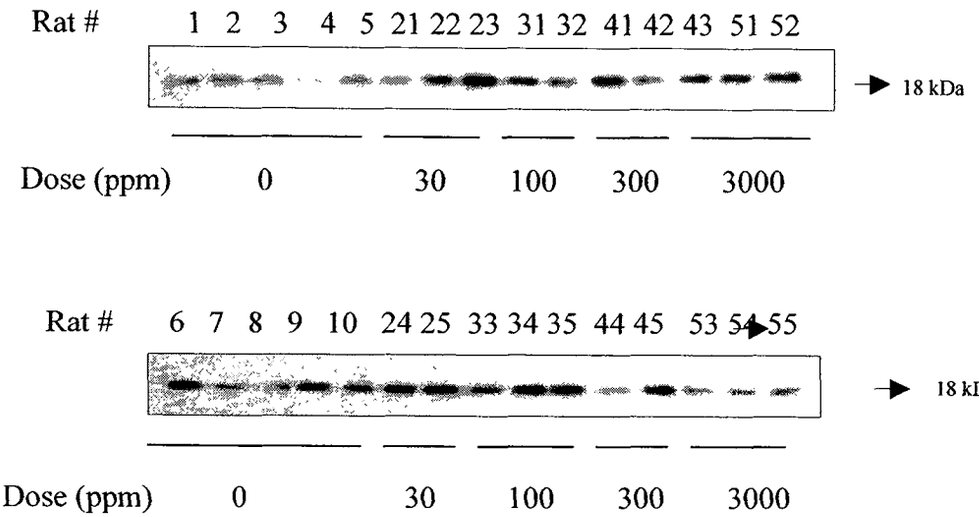
Female Kidney



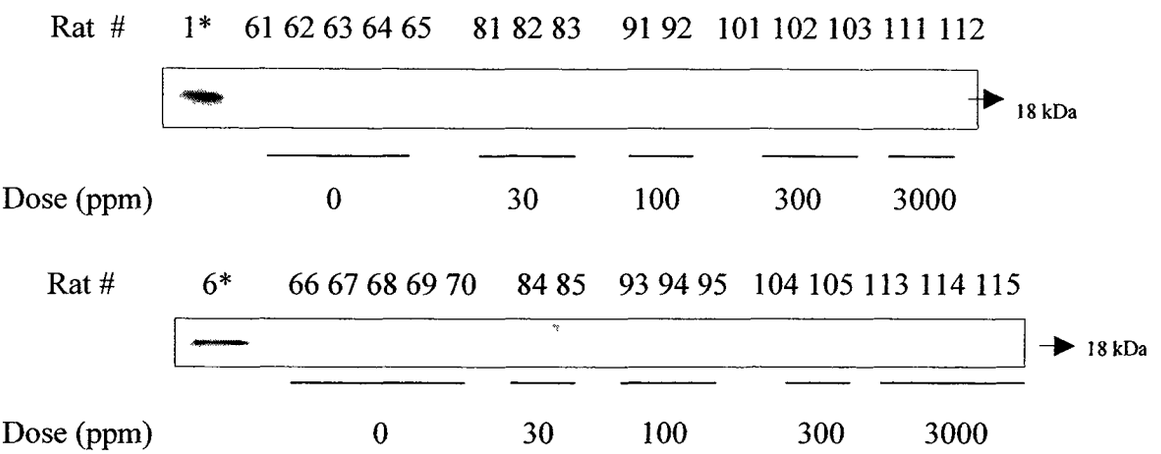
Male Rat Kidney samples numbers 1 and 6 were used as positive controls on female gels

Figure 4. α2u Globulin Expression in Livers of Male and Female Rats # 1-115

Male Liver



Female Liver



* Male Rat Liver samples numbers 1 and 6 were used as positive controls on female gels

Table 18. Quantitation of α2u Globulin Expression in Male Rat Liver and Kidney by Image Analysis.

Organ (isoform)	0ppm	30ppm	100ppm	300ppm	3000ppm
Liver (18.7kDa)	50.9±26.1 (100±51)	73.9±32.6 (145±64)	86.8±35.0 (171±68)	52.1±31.4 (102±62)	53.8±16.2 (106±32)
Kidney (16.2kDa)	35.3±23.0 (100±65)	20.7±10.0 (59±28)	45.3±16.1 (128±46)	49.5±31.2 (140±88)	30.7±14.5 (87±41)
Kidney (18.7kDa)	197.2±77.2 (100±39)	151.4±59.8 (77±30)	221.6±55.1 (112±28)	197.6±92.4 (100±47)	194.9±48.2 (99±24)

Values are expressed as dots/inch $x10^3$, Mean ± SD (% control).
n = 10 per group for the control group, and 5 per group for all test groups.
Kidney (16.2kDa), n = 8 per group for the control group, 3 per group for the 30ppm group, 4 per group for the 300ppm group, and 5 per group for the remaining test groups. A Student's t-test (2-sided) was performed on the results; * = statistically different from control $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

7. DISCUSSION

Cereclor S52 was administered in the diet to male and female Fischer 344 rats for 90 days. Dietary concentrations of 30, 100, 300 and 3000ppm were utilised. The resultant ingested dose levels were: 2.38, 9.34, 23.0 and 222 mg/kg body weight per day for male rats and 2.51, 9.70, 24.6 and 242 mg/kg body weight per day for female rats.

There were no dose-related effects on terminal bodyweight, body weight gain or food consumption. Absolute liver and kidney weights were increased only at the 3000ppm dose level in male and female rats.

No toxicologically significant effects were seen in the clinical chemistry data. Small, inconsistent changes in thyroid hormones were deemed not to be adverse, particularly since no concurrent histopathology was observed.

Minimal centrilobular hepatocyte hypertrophy was noted in the livers of male rats receiving 3000ppm Cereclor. This was not evident in male rats at the lower dose levels or in female rats at any dose. No treatment-related histopathology was reported in the kidneys or thyroids of male or female rats administered Cereclor S52 at dose levels of up to 3000ppm.

Based on these data we suggest a NOAEL of 300ppm for both male and female rats. In the current study, this equated to 23.0 and 24.6 mg/kg body weight for male and female rats respectively

8. REPORT

Once finalised the report will be issued to the Sponsor as follows:

One signed, bound copy; one photocopied, unbound copy.

Copies retained by CXR will be as follows:

One signed, unbound copy; one photocopied, unbound copy.

The Study Report has been prepared as described in CXR Biosciences SOP 0004. The histopathology report supplied by PHI Ltd., the immunocytochemistry report supplied by CCRM Biotech Ltd. and the individual animal data have been included as Appendices.

9. QUALITY ASSURANCE

Study activities at the Test Facility (CXR Biosciences Ltd.) and Test Sites (PHI Ltd. and CCRM Biotech Ltd.) have been conducted according to relevant SOPs.

No claim of GLP compliance is made for this study.

Study Protocol, data and procedures at the Test Facility, and the final draft of the Study Report, have been inspected according to CXR QA SOPs. These functions were carried out by personnel independent of the staff involved in the study.

QA inspections at the Test Site were conducted according to PHI SOPs. Copies of all inspection reports will be forwarded to the Study Director.

10. PROTOCOL DEVIATIONS

Vitamin K analysis was not done due to insufficient plasma samples.
The immunochemistry was performed at CCRMB Biotech Ltd, and not at PHI Ltd.
Plasma thyroid hormone assay results were read using equipment in the Dept. of Clinical Pharmacology and Therapeutics at Ninewells Hospital, Dundee.
Analyses of the 30ppm and 100ppm diet formulations were undertaken, with regard to concentration and homogeneity, by CXR Biosciences.
Control (0ppm) diet was stored, for a short period, at ambient temperature.
Environment enhancing bedding was added to all cages for the first week of the Study.

Details of these deviations, and their impact, have been documented in the Study File. None of these deviations is considered to affect the scientific integrity of this Study.

11. STUDY DIRECTORS STATEMENT

Final Report signature by the Study Director indicates acceptance of responsibility for the validity and integrity of the Study data.

12. QUALITY ASSURANCE STATEMENT

This Report has been audited by Quality Assurance and found to reflect the data generated for this study.
The following phases of the study were inspected by Quality Assurance at various stages during the study conduct, and were reported to the Study Director and to Management:

Phase of Study	**Date of Inspection**	**Date of Reporting**
Draft Protocol	11.8.04	11.8.04
Final Protocol	13.8.04	13.8.04

Study File
Data Recording System for Food Consumption

Study Report

J V Birnie, BSc, MIBiol, FRQA
Quality Assurance Auditor

Date

CXR Biosciences Ltd.
James Lindsay Place
DUNDEE DD1 5JJ

FINAL REPORT

STUDY TO INVESTIGATE THE ELIMINATION OF MEDIUM CHAIN CHLORINATED PARAFFINS IN MALE F344 RATS.

CXR STUDY NUMBER:	CXR0204
Study Director:	B M Elcombe
Sponsor:	Eurochlor Av. E. Van Nieuwehhuyse 4 Bt 2, B-1160 Brussels Belgium
Study Start Date:	1st March 2004
Study Finish Date:	24th June 2004

CIRCULATION

Signed Original:	Study File Sponsor
Copies to:	Study Director Sponsor

Author _____ 4th February 2005
Barbara Elcombe, Study Director — Date

Approved _____ 4th February 2005
Director, CXR Biosciences — Date

SUMMARY

Medium chain chlorinated paraffins (MCCPs) have been implicated in the failure of the clotting mechanism in rats. This study is one of a series of studies aimed to investigate MCCPs in the rat. Previous work has suggested that MCCPs are eliminated very slowly in the rat, but no definitive data exist. The aim of this study was to determine the elimination half-life of MCCP in the rat.

- The fat, skin-and-fur, liver and kidney contained the highest concentrations of radioactivity after a single gavage administration of ^{14}C-MCCP at 525mg/kg.
- Distribution into the liver and kidney was rapid, with highest levels seen 24 hours after dosing.
- Distribution into fat and skin-and-fur was slow with the highest levels seen in weeks 1 and 2 post-dose.
- After reaching a peak, elimination of radioactivity from the tissues occurred with an elimination half-life of approximately 2-5 days (well perfused tissues such as the liver) or approximately 2 weeks (poorly perfused tissues such as white adipose).
- Excretion *via* the faeces was the major route of elimination of radiolabelled material. Approximately 70% of the dose was recovered in the faeces and approximately 5% in the urine in the first 4 days after administration.
- As a maximum, only 50% (and mostly likely only 30%) of the administered ^{14}C-MCCP was absorbed.
- On completion of the study (88 days) approximately 2% of the administered radioactivity remained in the tissues.

CONTENTS

1. INTRODUCTION

Medium chain chlorinated paraffins (MCCPs) have been implicated in the failure of the clotting mechanism in rats. This study is one of a series of studies aimed to investigate MCCPs in the rat. Previous work has suggested that MCCPs are eliminated very slowly in the rat, but no definitive data exist. The aim of this study was to determine the elimination half-life of MCCP in the rat.

2. MATERIALS AND METHODS

2.1 Test Substance

[8-^{14}C]-Medium chain chlorinated paraffins (MCCPs), prepared by Blychem Ltd, Billingham TS23 4AZ, study no. 02BLY0137 (1g at 1.06mCi/g) and unlabelled Cereclor S52 (batch number 9695/1) were obtained from INEOS Chlor. Both test items were colourless liquids. Mazola pure corn oil was commercially freely available.

A formulation containing each test item in corn oil was prepared as follows:
1g of ^{14}C-MCCP plus 1.20833g of unlabelled Cereclor were made up to a total volume of 42ml with corn oil. This was a deviation from the protocol (as a result of the rats being larger than expected) giving a dosing solution of 52.5mg/ml (525mg/kg).

The formulation was stored at –20°C prior to dosing.

2.2 Safety Precautions

The normal safety precautions as detailed in the relevant SOP's and COSHH assessments applied. Additionally, the "Company Safe Working Procedures for Radioisotopes" was observed. No additional precautions were considered necessary.

2.3 Diet

RM1 diet (Special Diet Services Ltd., Stepfield, Witham, Essex, UK) was used. Specifications of the diet are available on request.

2.4 Animals

18 Male, 6-7 week old F344 rats were obtained from Harlan, UK. On arrival in the Medical School Resources Unit the rats were housed together in cages, 3 per cage on sawdust in solid-bottomed, polypropylene cages.

2.5 Animal Accommodation and Husbandry

In the animal room the environment was controlled to provide conditions suitable for the F344 strain of rat. The temperature was maintained within a range of 19-23°C and relative humidity within a range of 40-70%. There were a nominal 14-15 air changes per hour. Twelve-hour periods of light were cycled with twelve-hour periods of darkness.

Prior to the start, and for the duration of the study, the rats were allowed water and RM1 diet *ad libitum*.

3. EXPERIMENTAL DESIGN

The animals were uniquely numbered by ear-punch 2 to 5 days after arrival. At the start of the study an experimental card was placed on each cage showing the project licence code, treatment, study number, sex and individual number of the rat within, and identifying the Home Office Licensee. In addition, these cards were marked with radioactive tape to denote that the animals within had been treated with a radiochemical.

The study consisted of 18 male animals. Each animal was administered a single dose of ^{14}C-MCCP at 525mg/kg by gavage. The concentration of the ^{14}C-MCCP formulation was 52.5mg/ml of supplied chemical in corn oil (specific activity = 22μCi/ml), without any correction for purity. The volume of dosing solution administered was 10ml/kg bodyweight.

Three animals were sacrificed 24 hours after dosing, and blood and tissues collected according to section 5.6 "Terminal Procedures".

The remaining 15 animals were dosed on Day 1. 3 rats were immediately individually housed in glass metabolism cages, and 12 animals were housed together in individually numbered cages (3 per cage on sawdust in solid-bottomed, polypropylene cages).

From the 3 rats individually housed in glass metabolism cages, urine and faeces were collected once daily on Days 2 – 5 according to section 5.5 "Daily Procedures". The animals were sacrificed on Day 5 and blood, tissues and excreta collected according to section 5.6 "Terminal Procedures".

On Day 8 a second group of 3 rats were placed in glass metabolism cages and urine and faeces collected once daily on Days 9 - 12 according to section 5.5 "Daily Procedures". The animals were sacrificed on Day 12 and blood, tissues and excreta collected according to section 5.6 "Terminal Procedures".

On Day 22 another 3 rats were placed in metabolism cages and urine and faeces collected once daily on Days 23 - 26 according to section 5.5 "Daily Procedures". The animals were sacrificed on Day 26 and blood, tissues and excreta collected according to section 5.6 "Terminal Procedures".

On Day 50 another 3 rats were placed in metabolism cages and urine and faeces collected once daily on Days 51 - 54 according to section 5.5 "Daily Procedures". The animals were sacrificed on Day 54 and blood, tissues and excreta collected according to section 5.6 "Terminal Procedures".

On Day 85 the final 3 rats were placed in metabolism cages and urine and faeces collected once daily on Days 86 - 89 according to section 5.5 "Daily Procedures". The animals were sacrificed on Day 89 and blood, tissues and excreta collected according to section 5.6 "Terminal Procedures".

4. EXPERIMENTAL PROCEDURES

4.1 Radioactive Procedures

A "Contamination Monitoring Record" was kept, using a Geiger EP-15 to perform the monitoring. Monitoring of all surfaces took place before commencement and after completion of the study. After completion, a copy of the record was taken for the study file.

A "Contamination Monitoring Record" was kept for each cage. Monitoring of all cages took place before commencement and after completion of the study. After completion, a copy of the record was taken for the study file.

4.2 Clinical Observations

Prior to the start of the study, all rats were observed to ensure that they were physically normal and that they exhibited normal activity. All rats were judged to be normal.

4.3 Bodyweight

The bodyweight of each rat was recorded immediately before dosing at the start of the study. The animals were weighed weekly on the same day of the week. All animals were weighed prior to termination. The bodyweights were recorded in the study diary.

4.4 Daily Procedures

The individual urine and faeces collection pots were changed daily. The urine and faeces

collected from the treated rats were transferred, on ice, to CXR for analysis. Aliquots of the urine were placed in a -70°C freezer prior to analysis. The faeces were placed in a -70°C freezer prior to homogenisation and analysis.

4.5 Terminal Procedures

At the time of termination the rats were removed from the metabolic cages and killed by exposure to a rising concentration of CO_2.

4.5.1 Blood

Blood was taken by cardiac puncture into Lithium/Heparin coated tubes. The tubes were mixed immediately then a 0.5mL aliquot was taken from each tube, mixed with 0.5mL water and frozen at -70°C. The remainder was placed on ice prior to the preparation of plasma. After preparation, aliquots of the plasma were frozen at -70°C.

4.5.2 Tissues and organs

Liver, kidneys, lungs, heart, brain, testes and spleen were removed from each animal, weighed and flash frozen, in chunks, in liquid nitrogen. The thyroid/parathyroid and samples of muscle, skin and fat were removed from each animal and flash frozen, in chunks, in liquid nitrogen. The frozen tissues were transferred to CXR and placed in a -70°C freezer prior to analysis.

4.5.3 Excreta

The urine and faeces collected from the treated rats were transferred, on ice, to CXR for analysis. Aliquots of the urine were placed in a -70°C freezer prior to analysis. The faeces were placed in a -70°C freezer prior to homogenisation and analysis.

4.5.4 Carcasses

The remaining rat carcasses were double-wrapped and sealed in plastic bags before transfer, on ice, to CXR for storage at -70°C.

4.6 Radioactivity Measurements

Weighed samples of tissues and organs were digested in a proprietary solubiliser (Solvable) at up to 50°C overnight. Once digested, scintillant (Ultima Gold) was added. Strongly coloured samples were bleached with 30% hydrogen peroxide and then mixed with scintillant.

4.6.1 Plasma and blood

Blood

- 100µl blood (50:50 Blood:H_2O) was added to 1ml of Solvable.
- The mixture was incubated in a shaking waterbath at 50^0C, 82rev/min, for at least two hours.
- After cooling, 0.1ml of 0.1M EDTA was added.
- 0.5ml Hydrogen Peroxide was added and the mixture incubated in a shaking waterbath at 50^0C, 82rev/min, for 30 minutes.
- After cooling, 15ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Aliquots of any remaining sample were stored at -70°C awaiting potential further analysis.

Plasma

- 100µl plasma was added to 10ml "Ultima Gold" and allowed to stabilize (for both temperature and light) for at least one hour prior to counting.

Aliquots of any remaining sample were stored at -70°C awaiting potential further analysis.

4.6.2 Tissues and organs

Liver

- The weighed tissue sample was added to "Solvable" and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min overnight (until dissolved).
- After cooling overnight, 0.1ml of Hydrogen Peroxide was added, mixed, and the reaction allowed to subside.
- A further 0.1ml Hydrogen Peroxide was added, and the mixture was left for 30 minutes before being incubated in a shaking waterbath at 50^0C, 82rev/min, for 30 minutes.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Kidney

- The weighed tissue sample was added to "Solvable" and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min overnight (until dissolved)
- After cooling to room temperature, 0.1ml of Hydrogen Peroxide was added, mixed, and the reaction allowed to subside.

- A further 0.1ml Hydrogen Peroxide was added, and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min, for 30 minutes.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Lungs

- The weighed tissue sample was added to "Solvable" and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min overnight.
- After cooling to room temperature, 0.1ml of Hydrogen Peroxide was added, mixed, and the reaction allowed to subside.
- A further 0.1ml Hydrogen Peroxide was added, and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min, for 30 minutes.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Heart

- The weighed tissue sample was added to "Solvable" and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min overnight.
- After cooling to room temperature, 0.1ml of Hydrogen Peroxide was added, mixed, and the reaction allowed to subside.
- A further 0.1ml Hydrogen Peroxide was added, and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min, for 30 minutes.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Brain

- The weighed tissue sample was added to "Solvable" and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min overnight.
- After cooling to room temperature, 0.1ml of Hydrogen Peroxide was added, mixed, and the reaction allowed to subside.
- A further 0.1ml Hydrogen Peroxide was added, and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min, for 30 minutes.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Testes

- The weighed tissue sample was added to "Solvable" and the mixture incubated in a shaking waterbath at 50^0C, 82rev/min overnight.
- After cooling to room temperature, 0.1ml of Hydrogen Peroxide was added, mixed, and the reaction allowed to subside.

- A further 0.1ml Hydrogen Peroxide was added, and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min, for 30 minutes.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Spleen

- The weighed tissue sample was added to "Solvable" and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min overnight.
- After cooling to room temperature, 0.1ml of Hydrogen Peroxide was added, mixed, and the reaction allowed to subside.
- A further 0.1ml Hydrogen Peroxide was added, and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min, for 30 minutes.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Muscle

- The weighed tissue sample was added to "Solvable" and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min overnight.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Fat

- The weighed tissue sample was added to "Solvable" and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min overnight.
- After cooling to room temperature, 0.1ml of Hydrogen Peroxide was added, mixed, and the reaction allowed to subside.
- A further 0.1ml Hydrogen Peroxide was added, and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min, for 30 minutes.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Skin and Fur

- The weighed tissue sample was added to "Solvable" and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min overnight.
- After cooling to room temperature, 0.1ml of Hydrogen Peroxide was added, mixed, and the reaction allowed to subside.
- A further 0.1ml Hydrogen Peroxide was added, and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min, for 30 minutes.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

4.6.3 Excreta

Urine samples were added directly to scintillant. Strongly coloured samples were bleached with perchloric acid or hydrogen peroxide and then mixed with scintillant.
Faeces were homogenised prior to sampling for radioactive content to ensure that aliquots taken for analysis were representative of the bulk sample. The entire individual sample of faeces was weighed then homogenised, using a Polytron, in a known volume of phosphate buffered saline (PBS) to give a 10% homogenate. After homogenisation, an aliquot was digested in a proprietary solubiliser (Solvable) at up to 50°C overnight. Once digested the scintillant was added. Strongly coloured samples were bleached with 30% hydrogen peroxide and then mixed with scintillant. Aliquots of the remaining homogenate were stored at -70°C awaiting potential further analysis.

4.6.3.1 Urine

- 100µl urine was added to 10ml "Ultima Gold" and allowed to stabilize (for both temperature and light) for at least one hour prior to counting.

Aliquots of any remaining sample were stored at -70°C awaiting potential further analysis.

4.6.3.2 Faeces

- 500µl of the faecal homogenate sample was added to "Solvable" and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min overnight.
- After cooling to room temperature, 0.5ml sodium hypochlorite was added, and the mixture was incubated in a shaking waterbath at 50^0C, 82rev/min, for 60 minutes.
- After cooling, 10ml "Ultima Gold" was added and the whole mixture was allowed to stabilize for at least one hour prior to counting.

Aliquots of remaining sample were stored at -70°C awaiting potential further analysis.

4.6.4 Carcasses

Carcasses were kept frozen at -70°C awaiting the results of the radioactivity measurements in the removed tissues and organs.

5. RESULTS AND DISCUSSION

5.1 Specific Activity of the Dose Solution

The radioactivities measured in different volumes of dose solution are shown in Table 1 and Figure 1. Upon analysis, it is estimated that the specific activity of the dose solution was 0.48µCi/mg MCCP, having 25.2µCi/mL and 52.58mg MCCP/mL.

5.2 Bodyweight

The bodyweight of each rat immediately before dosing and prior to termination is reported in Table 2. The weekly animal weights are recorded in the study diary.

5.3 Intercurrent Deaths

Rat 13 was found dead during the study. There was no apparent reason for this death.

5.4 Tissue and Organ Weights

The individual tissue and organ weights of each rat, at termination, are reported in Table 2.

5.5 Radioactivity Measurements

5.5.1 Plasma, blood, tissues and organs

Individual plasma, blood, tissues and organs radioactivity measurements following gavage administration of ^{14}C-MCCP are shown in Table 3, Figure 2 and Appendix 1.

The animal numbers correspond to the following times post administration of ^{14}C-Cereclor

Rats 1-3 = 1 day
Rats 4-6 = 4 days ~1 week
Rats 7-9 = 11 days ~2 weeks
Rats 10-12 = 25 days ~4 weeks
Rats 13-15 = 53 days ~8 weeks
Rats 16-18 = 88 days ~13 weeks

At termination 24 hours after administration of ^{14}C-MCCP (rats 1-3), the liver, kidney, fat and skin-and-fur contained the highest concentrations of radioactivity. Thereafter, concentrations of radioactivity declined in all tissues except the fat and the skin-and-fur. At termination 96 hours after administration of ^{14}C-MCCP (rats 4-6), concentrations of radioactivity in the fat and skin-and-fur were substantially higher than in the other tissues. In the fat and skin-and-fur concentrations peaked 1 to 2 weeks after administration of the compound. In all sample types, after achieving a maximum, the concentration of radioactivity declined but was still measurable at the end of the study (week 13).

Mean tissue distribution of radioactivity as a percentage of the administered dose is shown in Table 4, Figure 3. The amount of dose recovered from the tissues remained at approximately 7% during the first 2 weeks of the study. Thereafter, the percentage

of the dose recovered from the tissues declined to a value of approximately 2% by week 13. Initially, the liver contained approximately 1.6% of the administered radioactivity but this rapidly declined, and at 96 hours only 0.3% of the administered dose was recovered from the liver. At all sampling times after 24 hours, the fat, muscle and skin-and-fur contained most of the dose recovered from the body.

Mean tissue:plasma distribution ratios of ^{14}C-MCCP in the male rat after gavage administration of ^{14}C-MCCP at 525mg/kg are shown in Table 5. The mean ratio tissue:plasma remained constant in most tissues throughout the study, suggesting good equilibrium between the plasma and these tissues. In general, these were the well-perfused tissues. The distribution between the plasma and fat and skin-and-fur was not constant over time. In these two tissues, radioactivity appeared to accumulate over the first 2 weeks after dosing. Thereafter, the amount of radioactivity in these tissues relative to the plasma declined but remained substantially higher than that in the other tissues at all times post dose. This suggests that the radioactivity was gradually released back into the systemic circulation from these tissues and presumably eliminated from the body. Over time there was an increase in the blood:plasma distribution of radioactivity, suggesting slower elimination from the red cells than from the plasma.

Figure 4 illustrates the time-dependent redistribution and elimination of ^{14}C-MCCP in the blood, plasma, liver kidney, white adipose tissue and skin-and-fur. The approximate half-life of ^{14}C-MCCP in blood, plasma and well-perfused tissues (liver, kidney) was 2-5days. In poorly perfused tissues, such as adipose, the approximate half-life was 2 weeks.

5.5.2 Urine and faeces

The percentage of the administered dose recovered in the urine and faeces of the male rat following gavage administration of ^{14}C-MCCP at 525mg/kg are shown in Table 6 and Figures 5 and 6. Individual values are shown in Appendix 2.

These data show that the primary route of elimination of ^{14}C-MCCP was via the faeces. A large proportion of the administered dose (~50%) was rapidly eliminated *via* this route. It is reasonable to assume that the radioactivity measured at 24 hours post dose represents material that was not absorbed, therefore, a maximum of only 50% of the dose was absorbed after gavage administration of ^{14}C-MCCP at 525mg/kg. At termination 96 hours after administration of ^{14}C-MCCP, there was 71% of the administered dose eliminated *via* the faeces and 6% of the dose eliminated *via* the urine. This suggests that only about 30% of the orally administered dose of ^{14}C-MCCP was absorbed.

Radioactivity remained measurable in the urine and faeces for the 13 week duration of the study. It was not possible to collect all urine and faeces samples over this time but it is reasonable to assume this accounted for the bulk of the material eliminated.

5.5.3 Mass balance

This study was not designed as a mass balance study, however the percentage dose recovered in urine, faeces and tissues of male rats following gavage administration of ^{14}C-MCCP at 525mg/kg are shown in Table 7. Cage-washings, residual radioactivity within the gut and bladder, and tissues (particularly fatty tissues such as the bone marrow) not sampled at termination, were not recorded. Only rats 4, 5 and 6 were kept in metabolic cages immediately post dose until termination. In these animals the average total recovery of radioactivity from those tissues and organs sampled, without cage-washings, gut and bladder, was 83.6% of the administered dose.

6. CONCLUSION

The results indicate that, in the male rat, a maximum of only approximately 50% (and most probably only 30%) of the administered ^{14}C-MCCP was absorbed and this was widely distributed. The total recovery of radiolabelled material after 96hours was high (83.6%). The faeces were the major route of elimination of radiolabelled material, 71.4% being recovered after 96 hours.

Approximately 7% of the administered dose was recovered from plasma, blood, tissues and organs 24 hours after dosing. In the body at this time, radioactivity was distributed primarily in the skin-and-fur (2.7%), liver (1.6%), muscle (1.3%) and fat (0.8%).

The amount of radioactivity (as % dose) recovered from the body at 24 hours, 96 hours and 2 weeks after dosing remained approximately the same (7%) however there was a redistribution into fat (a maximum of 2.5 % in week 2) and skin-and-fur (a maximum of 3.7% in week 2) over this time.

Elimination of radioactivity from the tissues was seen throughout the study, with approximately 2% of the administered dose remaining, primarily in the skin-and-fur, at week 13.

The half-life of ^{14}C-MCCP in well-perfused tissues was approximately 2-5 days and in poorly perfused tissues was about 2 weeks.

7. RAW DATA STORAGE

Experimental details and raw data from the study shall be stored in the Study File and archived along with copies of the Draft Report(s) and/or Final Report (collectively the "Archived Materials") for a period of two (2) years from the date of issue of the Final Report without charge (the "Retention Period") at CXR's designated archive. At the expiry

of the Retention Period in the absence of further agreement for retention by CXR at the Sponsor's expense, Archived Materials will be returned to the Sponsor at the Sponsor's risk and expense unless the Sponsor instructs CXR to destroy the same which destruction shall be at the Sponsor's expense. During the Retention Period CXR shall provide access to the Client to the Archived Materials and the Client shall have the right to copy the same, at the Client's expense. Sponsor's access to the archived items will be provided in accordance with CXR Archive SOP#0007.

8. TABLES AND FIGURES

Table 1: Radioactivity measurements per unit volume of dosing solution

Correlation coeff		0.99955781	Diln.Factor: 1
Intercept		-0.04033127	
Slope		0.04892254	

µL dosing sol	Dpm x 10^{-6}	Y Calc.
0	0.000	-0.040
10	0.468	0.449
20	0.926	0.938
50	2.309	2.406
100	4.901	4.852

Figure 1: Radioactivity measurements per unit volume of dosing solution

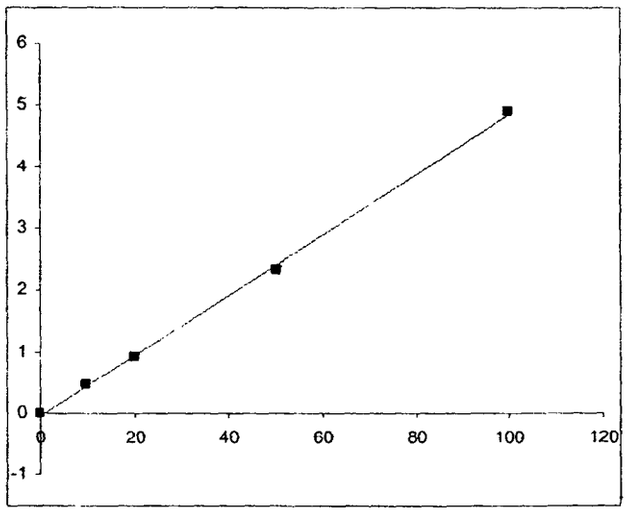


Table 2: Individual Body, Organ and Tissue Weights (g)

	Initial Bwt	Terminal Bwt	Dose (dpm x 10*6)	Plasma	Blood	Liver	L. Kidney	R. Kidney	Total Kidney	Spleen	Lungs	Heart	Brain	L. Testis	R. Testis	Total Testes	Fat	Muscle	Skin + Fur
Rat 1	191	183.2	92.19	5.734	10.626	7.941	0.757	0.801	1.558	0.446	0.884	0.606	1.773	1.212	1.241	2.453	7.328	91.600	29.312
Rat 2	191	182.03	92.19	5.698	10.558	7.295	0.591	0.410	1.001	0.309	0.520	0.400	1.536	1.079	1.910	2.989	7.281	91.015	29.125
Rat 3	226	212.29	111.60	6.645	12.313	7.250	0.567	0.518	1.085	0.213	0.778	0.453	1.602	1.156	1.059	2.215	8.492	106.145	33.966
Rat 4	210	201.12	101.89	6.295	11.665	9.112	0.834	0.519	0.853	0.141	0.840	0.332	1.549	1.420	1.157	2.577	8.045	100.560	32.179
Rat 5	209	211.16	101.89	6.609	12.247	7.717	0.740	0.747	1.487	0.418	1.143	0.568	1.754	1.340	1.290	2.630	8.446	105.580	33.786
Rat 6	217	217.38	106.74	6.804	12.608	9.208	0.844	0.795	1.639	0.691	1.212	0.737	1.763	1.435	1.393	2.828	8.695	108.690	34.781
Rat 7	244	248	116.45	7.762	14.384	8.899	0.826	0.849	1.675	0.550	0.965	0.741	1.854	1.413	1.322	2.735	9.920	124.000	39.680
Rat 8	219	237	106.74	7.418	13.746	9.456	0.826	0.835	1.661	0.463	1.014	0.714	1.897	1.365	1.347	2.712	9.480	118.500	37.920
Rat 9	210	215	101.89	6.730	12.470	7.493	0.762	0.746	1.508	0.430	0.973	0.661	1.784	1.382	1.322	2.704	8.600	107.500	34.400
Rat 10	219	231.27	106.74	7.239	13.414	7.816	0.775	0.686	1.461	0.435	0.978	0.647	1.847	1.242	1.195	2.437	9.251	115.635	37.003
Rat 11	219	252.33	106.74	7.898	14.635	9.181	0.842	0.829	1.671	0.468	1.107	0.787	1.946	1.492	1.490	2.982	10.093	126.165	40.373
Rat 12	209	247.24	101.89	7.739	14.340	9.002	0.839	0.872	1.711	0.523	1.069	0.733	1.857	1.445	1.364	2.809	9.890	123.620	39.558
Rat 13	205		99.50																
Rat 14	247	314.89	121.30	9.856	18.264	11.930	1.056	1.025	2.081	0.658	1.182	0.937	1.957	1.652	1.531	3.183	12.596	157.445	50.382
Rat 15	218	265.5	106.74	8.310	15.399	7.675	0.877	0.851	1.728	0.480	1.024	0.804	1.894	1.389	1.346	2.735	10.620	132.750	42.480
Rat 16	228	298.67	111.60	9.348	17.323	6.990	0.946	0.937	1.883	0.516	1.280	1.000	1.901	1.494	1.514	3.008	11.947	149.335	47.787
Rat 17	227	304.43	111.60	9.529	17.657	7.535	0.922	0.963	1.885	0.573	1.366	0.950	2.007	1.611	1.567	3.178	12.177	152.215	48.709
Rat 18	197	286.53	97.04	8.968	16.619	7.698	0.999	0.982	1.981	0.517	1.348	0.959	2.022	1.499	1.438	2.937	11.461	143.265	45.845

Blood volume based on 58mL/kg body wt page 1276 handbook of Toxicology
Plasma volume based on 31.3mL/kg body wt, page 1276, handbook of Toxicology
Muscle mass based on physiological parameters organ volumes muscle is 50% of body mass
Fat mass is based on physiological parameters, organ volumes, fat is 4% of body mass.
Skin and fur was taken as 16% of body mass.

Table 3: Tissue radioactivity measurements (dpm/mg or μl) in the male rat following gavage administration of ^{14}C-MCCP at 525mg/kg

Animal	Plasma	Blood	Liver	L. Kidney	R. Kidney	Spleen	Lungs	Heart	Brain	L. Testis	R. Testis	Fat	Muscle	Skin + Fur
1	20.84	11.12	175.40	48.10	71.65	23.79	26.75	17.77	12.23	2.46	13.05	109.33	16.46	91.26
2	28.67	10.72	199.50	55.58	42.92	32.97	41.27	21.80	14.91	15.37	14.75	108.29	10.07	116.06
3	30.23	16.32	266.60	61.47	59.90	31.91	35.55	28.57	19.02	15.30	15.20	82.47	13.50	52.15
Mean	**26.58**	**12.72**	**213.83**	**55.05**	**58.16**	**29.56**	**34.52**	**22.71**	**15.39**	**11.05**	**14.33**	**100.03**	**13.34**	**86.49**
SD	**5.03**	**3.12**	**47.26**	**6.70**	**14.44**	**5.02**	**7.31**	**5.46**	**3.42**	**7.44**	**1.14**	**15.22**	**3.20**	**32.22**
4	4.80	7.52	30.70	36.39	27.45	15.37	15.46	9.47	6.24	6.96	7.56	138.55	11.18	97.28
5	7.12	7.54	46.80	43.17	66.11	16.17	18.98	11.38	7.55	9.32	9.28	137.34	6.67	127.19
6	5.33	4.58	32.30	19.93	20.00	13.86	13.63	9.39	4.34	11.34	5.84	102.37	13.71	8.10 *
Mean	**5.75**	**6.55**	**36.60**	**33.16**	**37.85**	**15.13**	**16.02**	**10.08**	**6.04**	**9.21**	**7.56**	**126.09**	**10.52**	**112.24**
SD	**1.22**	**1.70**	**8.87**	**11.95**	**24.75**	**1.17**	**2.72**	**1.13**	**1.61**	**2.19**	**1.72**	**20.55**	**3.57**	**21.15**
7	2.54	3.96	14.60	14.77	14.88	9.72	22.47	9.28	4.56	5.78	5.40	224.34	10.84	103.35
8	2.56	4.06	30.50	17.59	17.37	9.35	20.66	9.71	4.72	4.79	6.20	303.38	7.52	124.03
9	3.13	3.38	24.30	19.54	18.04	12.55	16.71	10.55	4.75	5.64	5.77	333.01	19.59	94.87
Mean	**2.74**	**3.80**	**23.13**	**17.30**	**16.77**	**10.54**	**19.95**	**9.85**	**4.68**	**5.40**	**5.79**	**286.91**	**12.65**	**107.42**
SD	**0.34**	**0.37**	**8.01**	**2.40**	**1.67**	**1.75**	**2.95**	**0.65**	**0.10**	**0.54**	**0.40**	**56.18**	**6.24**	**15.00**
10	1.10	2.10	5.20	5.40	5.52	3.87	5.00	3.54	2.68	2.50	3.12	125.01	3.91	33.33
11	1.73	2.70	9.50	9.55	9.22	6.24	9.07	4.76	3.75	4.13	11.72	211.89	4.25	82.99
12	0.90	2.12	5.00	5.20	4.99	4.30	3.99	3.19	2.53	2.14	2.37	162.43	3.14	35.09
Mean	**1.24**	**2.31**	**6.57**	**6.72**	**6.58**	**4.80**	**6.02**	**3.83**	**2.99**	**2.93**	**5.73**	**166.44**	**3.77**	**50.47**
SD	**0.43**	**0.34**	**2.54**	**2.46**	**2.31**	**1.26**	**2.69**	**0.82**	**0.67**	**1.06**	**5.20**	**43.58**	**0.57**	**28.18**
13														
14	0.68	1.60	2.40	5.93	4.17	2.70	3.59	1.76	1.85	1.71	1.44	86.44	1.50	7.48
15	0.50	2.00	2.21	3.68	2.31	2.30	3.66	1.41	2.68	1.14	0.89	62.23	1.63	9.77
Mean	**0.59**	**1.80**	**2.31**	**4.80**	**3.24**	**2.50**	**3.63**	**1.59**	**2.27**	**1.43**	**1.16**	**74.34**	**1.57**	**8.63**
SD	**0.13**	**0.28**	**0.13**	**1.59**	**1.32**	**0.28**	**0.05**	**0.25**	**0.59**	**0.40**	**0.39**	**17.12**	**0.09**	**1.62**
16	0.66	3.60	4.71	4.58	3.19	3.24	3.32	2.43	3.10	1.93	2.89	69.69	2.46	9.09
17	0.68	3.82	3.87	7.07	3.46	2.89	2.89	2.36	2.53	3.12	1.42	55.88	2.19	16.14
18	0.66	3.72	5.18	5.57	5.65	3.02	2.62	2.26	2.90	1.55	5.33	62.33	2.91	8.29
Mean	**0.67**	**3.71**	**4.59**	**5.74**	**4.10**	**3.05**	**2.94**	**2.35**	**2.84**	**2.20**	**3.21**	**62.63**	**2.52**	**11.17**
SD	**0.01**	**0.11**	**0.66**	**1.25**	**1.35**	**0.18**	**0.35**	**0.09**	**0.29**	**0.82**	**1.97**	**6.91**	**0.36**	**4.32**

* Unusually low result not included in the calculations.

Table 4. Individual Animal Data - % of administered dose recovered from each total organ / tissue

Rat no.	Plasma	Blood	Liver	Total Kidney	Spleen	Lungs	Heart	Brain	Total Testes	Fat	Muscle	Skin+Fur	Total
1	0.130	0.128	1.511	0.102	0.012	0.026	0.012	0.024	0.021	0.869	1.636	2.901	
2	0.177	0.123	1.579	0.055	0.011	0.023	0.009	0.025	0.049	0.855	0.994	3.666	
3	0.180	0.180	1.732	0.059	0.006	0.025	0.012	0.027	0.030	0.627	1.284	1.587	
mean	**0.162**	**0.144**	**1.607**	**0.072**	**0.010**	**0.025**	**0.011**	**0.025**	**0.033**	**0.784**	**1.305**	**2.718**	**6.895**
sd	**0.028**	**0.032**	**0.113**	**0.026**	**0.003**	**0.001**	**0.001**	**0.002**	**0.014**	**0.136**	**0.321**	**1.051**	**1.729**
4	0.030	0.086	0.275	0.044	0.002	0.013	0.003	0.009	0.018	1.093	1.103	3.072	
5	0.046	0.091	0.354	0.080	0.007	0.021	0.006	0.013	0.024	1.139	0.691	4.218	
6	0.034	0.054	0.279	0.031	0.009	0.015	0.006	0.007	0.023	0.834	1.396	0.264 *	
mean	**0.037**	**0.077**	**0.303**	**0.051**	**0.006**	**0.017**	**0.005**	**0.010**	**0.022**	**1.022**	**1.064**	**3.645**	**6.258**
sd	**0.009**	**0.020**	**0.045**	**0.025**	**0.003**	**0.004**	**0.002**	**0.003**	**0.003**	**0.164**	**0.354**	**0.810**	**1.443**
7	0.017	0.049	0.112	0.021	0.005	0.019	0.006	0.007	0.013	1.911	1.154	3.522	
8	0.018	0.052	0.270	0.027	0.004	0.020	0.006	0.008	0.014	2.694	0.835	4.406	
9	0.021	0.041	0.179	0.028	0.005	0.016	0.007	0.008	0.015	2.811	2.067	3.203	
mean	**0.018**	**0.048**	**0.187**	**0.025**	**0.005**	**0.018**	**0.006**	**0.008**	**0.014**	**2.472**	**1.352**	**3.710**	**7.864**
sd	**0.002**	**0.006**	**0.080**	**0.004**	**0.001**	**0.002**	**0.000**	**0.001**	**0.001**	**0.489**	**0.639**	**0.623**	**1.847**
10	0.007	0.026	0.038	0.007	0.002	0.005	0.002	0.005	0.006	1.083	0.424	1.154	
11	0.013	0.037	0.082	0.015	0.003	0.009	0.004	0.007	0.022	2.003	0.502	3.139	
12	0.007	0.030	0.044	0.009	0.002	0.004	0.002	0.005	0.006	1.577	0.381	1.362	
mean	**0.009**	**0.031**	**0.055**	**0.010**	**0.002**	**0.006**	**0.003**	**0.005**	**0.012**	**1.554**	**0.436**	**1.885**	**4.008**
sd	**0.003**	**0.005**	**0.024**	**0.004**	**0.001**	**0.003**	**0.001**	**0.001**	**0.009**	**0.460**	**0.062**	**1.091**	**1.663**
13													
14	0.006	0.023	0.024	0.009	0.001	0.003	0.001	0.003	0.004	0.898	0.195	0.311	
15	0.004	0.028	0.016	0.005	0.001	0.004	0.001	0.005	0.003	0.619	0.203	0.389	
mean	**0.005**	**0.026**	**0.020**	**0.007**	**0.001**	**0.004**	**0.001**	**0.004**	**0.003**	**0.759**	**0.199**	**0.350**	**1.377**
sd	**0.001**	**0.003**	**0.005**	**0.003**	**0.000**	**0.000**	**0.000**	**0.001**	**0.001**	**0.197**	**0.006**	**0.055**	**0.274**
16	0.006	0.056	0.030	0.007	0.001	0.004	0.002	0.005	0.007	0.746	0.329	0.389	
17	0.006	0.060	0.026	0.009	0.001	0.004	0.002	0.005	0.006	0.610	0.299	0.704	
18	0.006	0.064	0.041	0.011	0.002	0.004	0.002	0.006	0.010	0.736	0.430	0.392	
mean	**0.006**	**0.060**	**0.032**	**0.009**	**0.002**	**0.004**	**0.002**	**0.005**	**0.008**	**0.697**	**0.353**	**0.495**	**1.672**
sd	**0.000**	**0.004**	**0.008**	**0.002**	**0.000**	**0.000**	**0.000**	**0.001**	**0.002**	**0.076**	**0.068**	**0.181**	**0.344**

Figure 2. Tissue radioactivity measurements (dpm/mg or µl) in the male rat following gavage administration of ^{14}C-MCCP at 525mg/kg

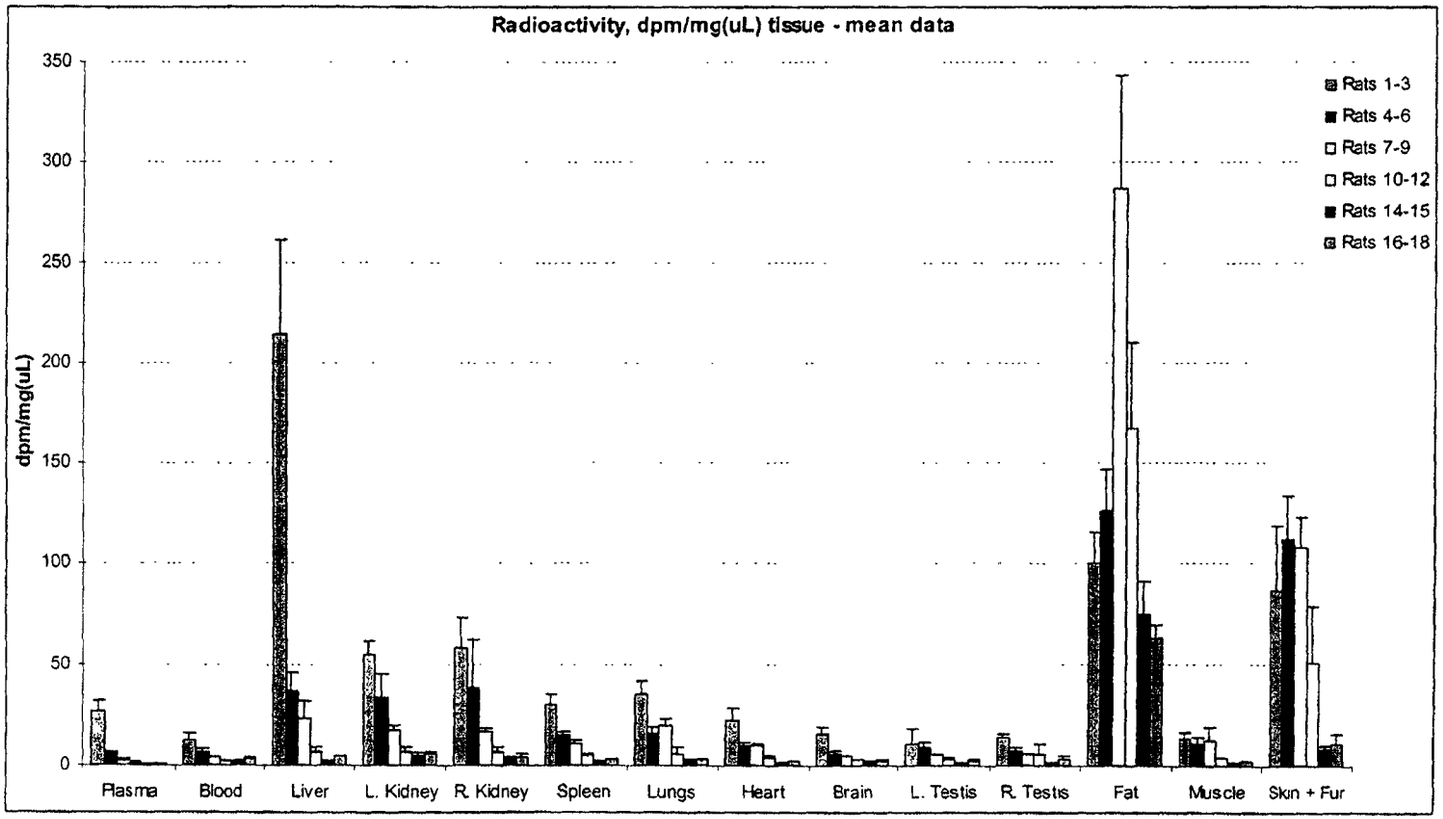


Figure 3. % of administered dose recovered from each total organ / tissue

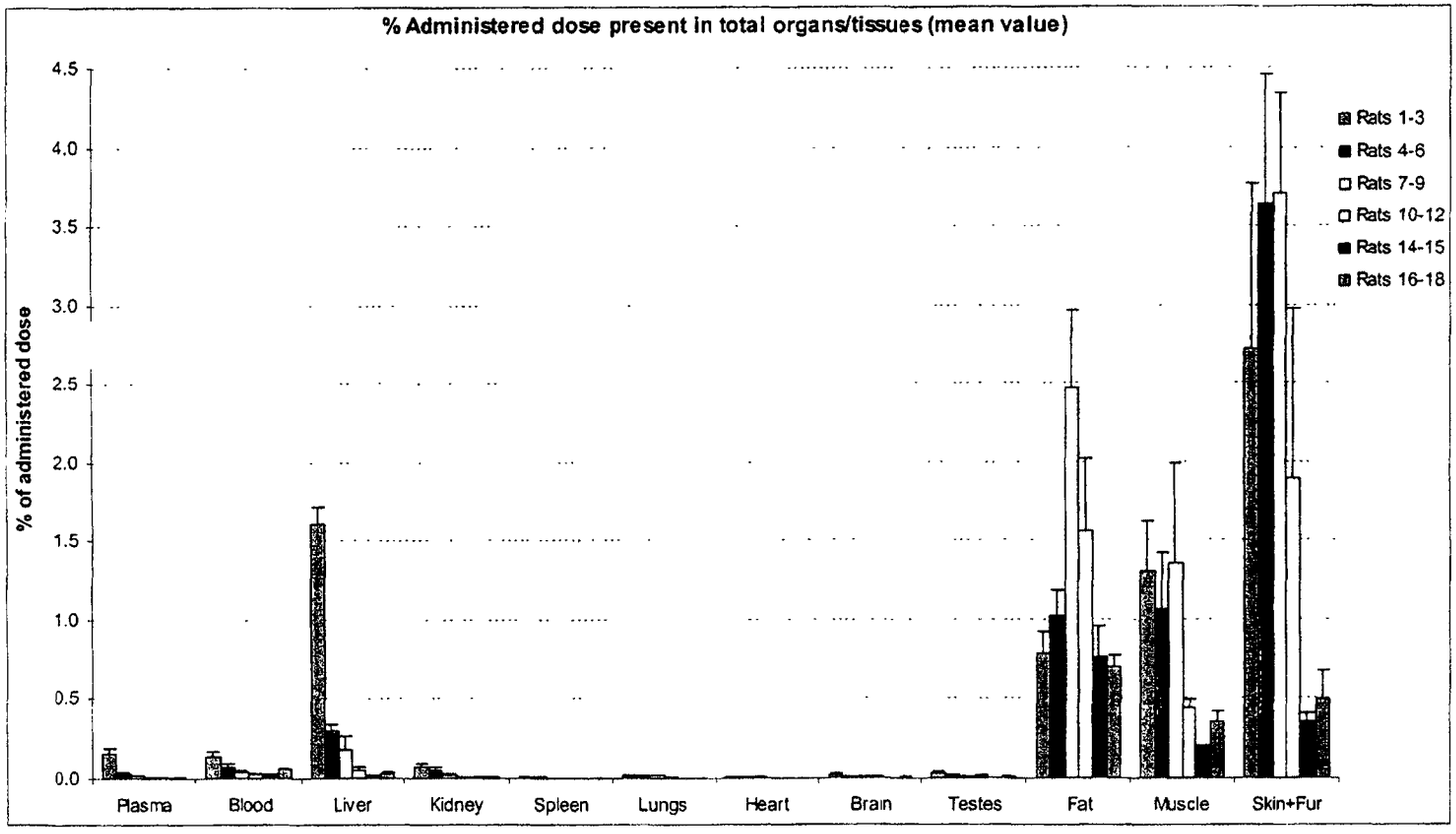
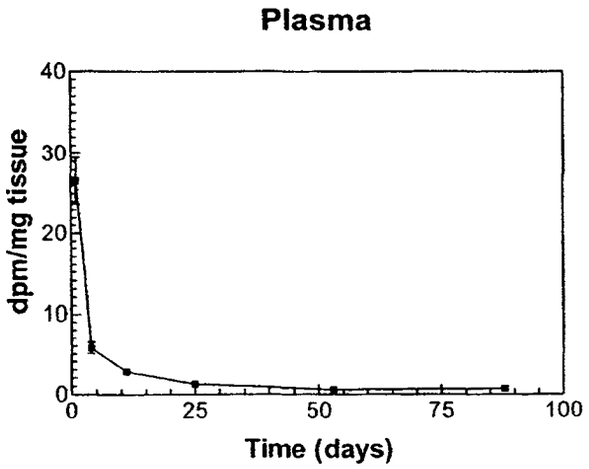


Table 5. Mean tissue:plasma distribution ratio in the male rat following gavage administration of ^{14}C-MCCP at 525mg/kg

Animals	Plasma	Blood	Liver	L. Kidney	R. Kidney	Spleen	Lungs	Heart	Brain	L. Testis	R. Testis	Fat	Muscle	Skin + Fur
Rats 1-3	1	0.479	8.045	2.071	2.188	1.112	1.299	0.855	0.579	0.416	0.539	3.763	0.502	3.254
Rats 4-6	1	1.139	6.365	5.768	6.583	2.632	2.787	1.753	1.051	1.602	1.314	21.928	1.830	19.519
Rats 7-9	1	1.385	8.433	6.306	6.111	3.842	7.271	3.589	1.705	1.968	2.110	104.584	4.611	39.156
Rats 10-12	1	1.855	5.282	5.404	5.290	3.863	4.842	3.080	2.402	2.353	4.612	133.869	3.029	40.592
Rats 14-15	1	3.051	3.907	8.144	5.494	4.237	6.144	2.686	3.839	2.420	1.970	125.992	2.653	14.627
Rats 16-18	1	5.570	6.880	8.608	6.149	4.575	4.415	3.525	4.265	3.299	4.819	93.950	3.780	16.755

Figure 4. Elimination of ^{14}C-MCCP from major tissues of the male rat.



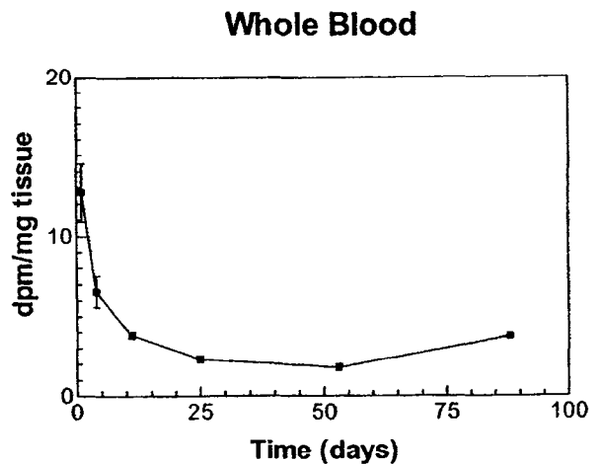
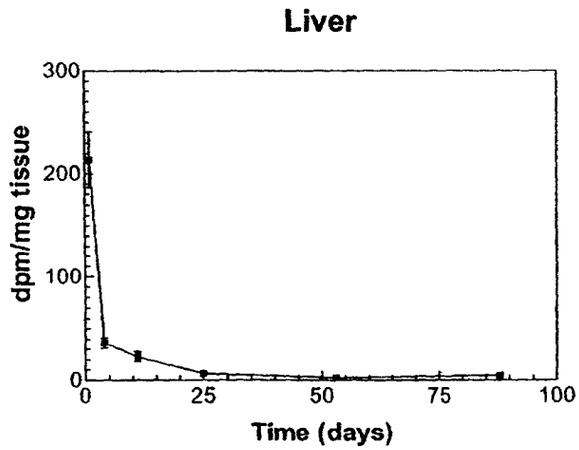


Figure 4 continued. Elimination of ^{14}C-MCCP from major tissues of the male rat.



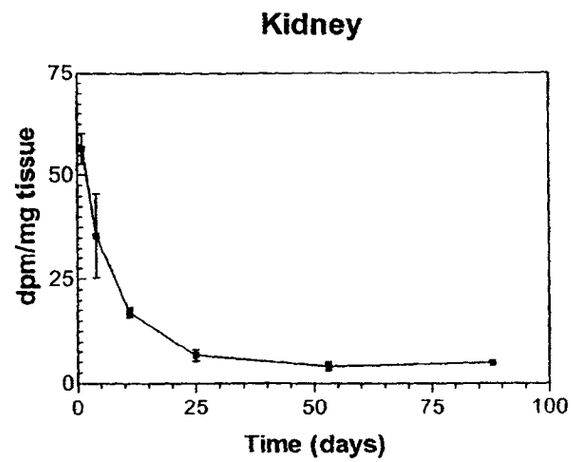
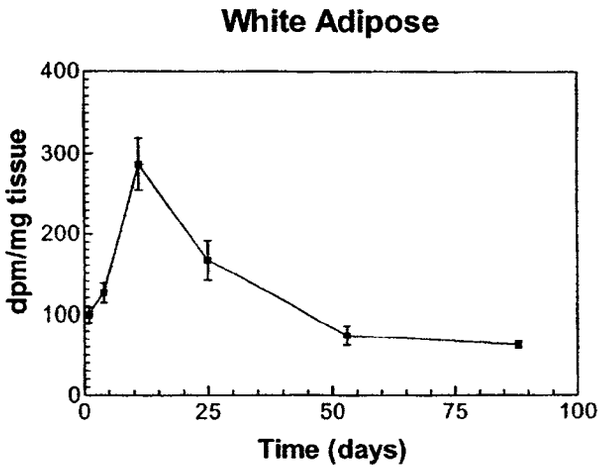


Figure 4 continued. Elimination of ^{14}C-MCCP from major tissues of the male rat.



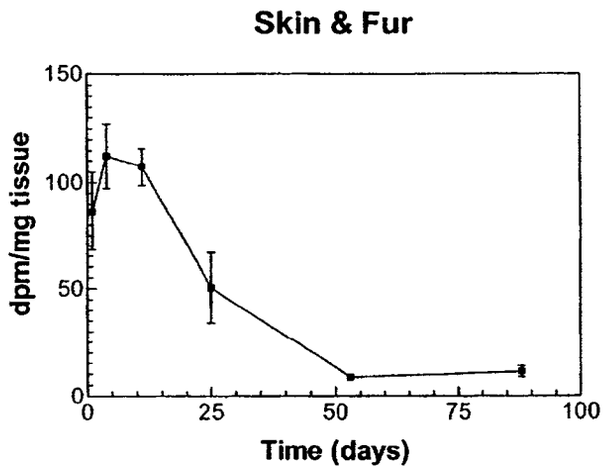


Table 6. Mean Data - % of administered dose recovered from urine and faeces of the male rat following gavage administration of ^{14}C-MCCP at 525mg/kg

Rat numbers	Days post-dosing	Total Urine dpm, mean	sd	Urine % dose	sd	Total	Total Faeces dpm, mean	sd	Faeces % dose	sd	Total
4-6	1	4624398.73	2407611.18	4.43	2.17		51683953.76	20605477.70	49.72	19.12	
	2	1111156.93	442336.79	1.08	0.45		19238514.09	13603339.24	18.73	13.50	
	3	326929.13	49719.81	0.32	0.06		2447726.65	1366149.54	2.39	1.36	
	4	122519.83	15206.34	0.12	0.02	5.94	610717.10	296132.54	0.59	0.30	71.44
7-9	8	50385.30	2200.99	0.05	0.00		96202.01	24834.19	0.09	0.02	
	9	82481.97	22953.52	0.08	0.02		210046.40	30975.98	0.19	0.03	
	10	54317.83	24476.29	0.05	0.03		171454.71	63038.02	0.16	0.06	
	11	39776.23	8764.30	0.04	0.01	0.21	148000.40	14891.60	0.14	0.02	0.58
10-12	22	15853.00	6882.25	0.02	0.01		44070.02	6914.92	0.04	0.01	
	23	22509.82	8050.88	0.02	0.01		43330.03	31950.00	0.04	0.03	
	24	16282.47	8515.23	0.02	0.01		39926.52	13157.62	0.04	0.01	
	25	13838.50	1369.43	0.01	0.00	0.06	53423.08	20548.92	0.05	0.02	0.17
14-15	50	7252.55	2184.89	0.01	0.00		11287.75	2657.36	0.01	0.00	
	51	8769.15	798.11	0.01	0.00		8733.18	9165.46	0.01	0.01	
	52	7732.75	407.65	0.01	0.00		10796.86	5365.64	0.01	0.00	
	53	9775.25	2106.12	0.01	0.00	0.03	25652.15	8705.84	0.02	0.01	0.05
16-18	85	7362.87	1166.77	0.01	0.00		2340.09	1737.80	0.00	0.00	
	86	8228.47	2066.28	0.01	0.00		9365.92	5000.09	0.01	0.00	
	87	6615.40	1686.84	0.01	0.00		3505.07	2095.59	0.00	0.00	
	88	7526.33	1528.51	0.01	0.00	0.03	2878.13	2760.88	0.00	0.00	0.02

Figure 5. Mean Data - % of administered dose recovered from urine and faeces of the male rat following gavage administration of ^{14}C-MCCP at 525mg/kg

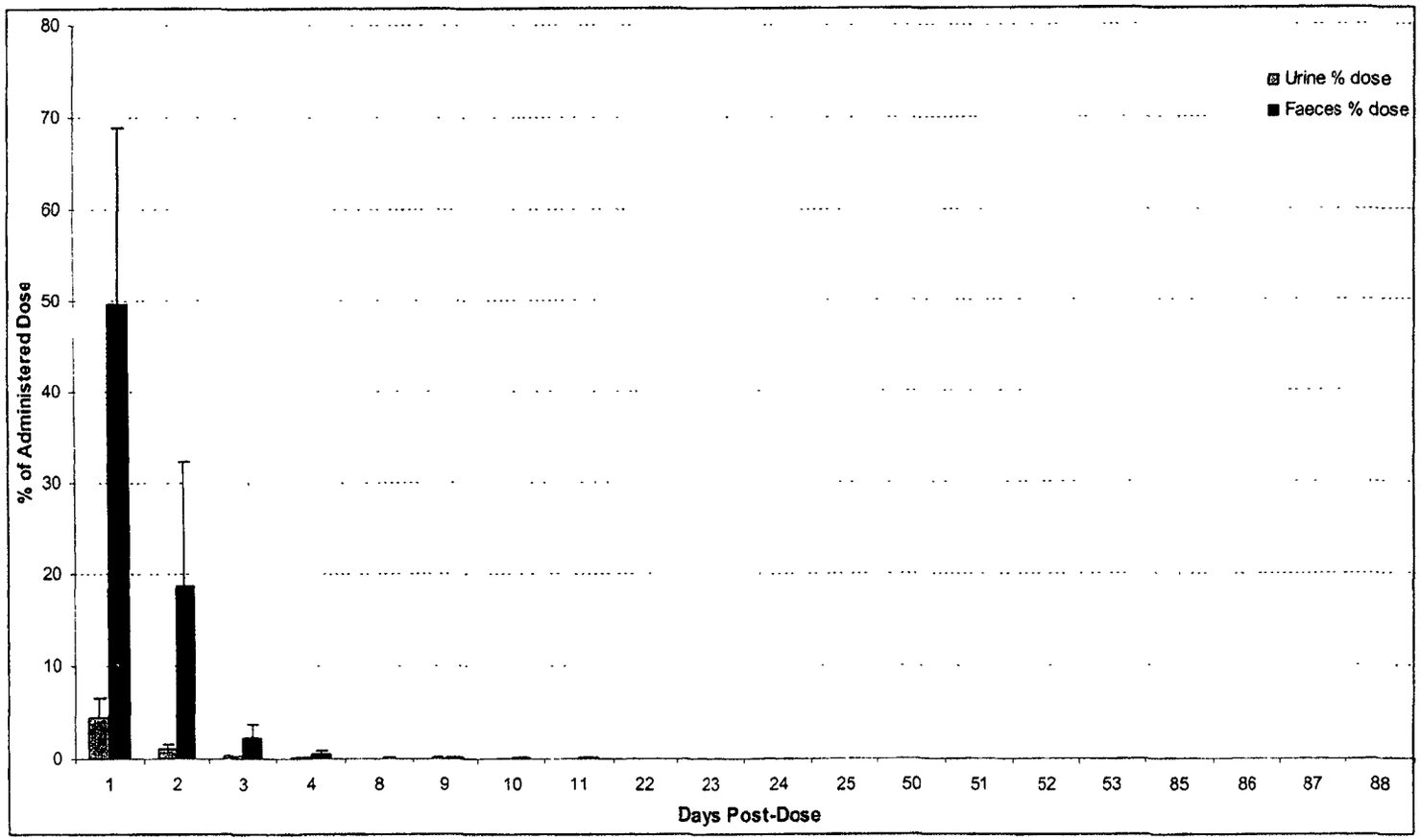


Figure 6. Mean Data - % of administered dose recovered from urine and faeces of the male rat following gavage administration of ^{14}C-MCCP at 525mg/kg, excluding week 1.

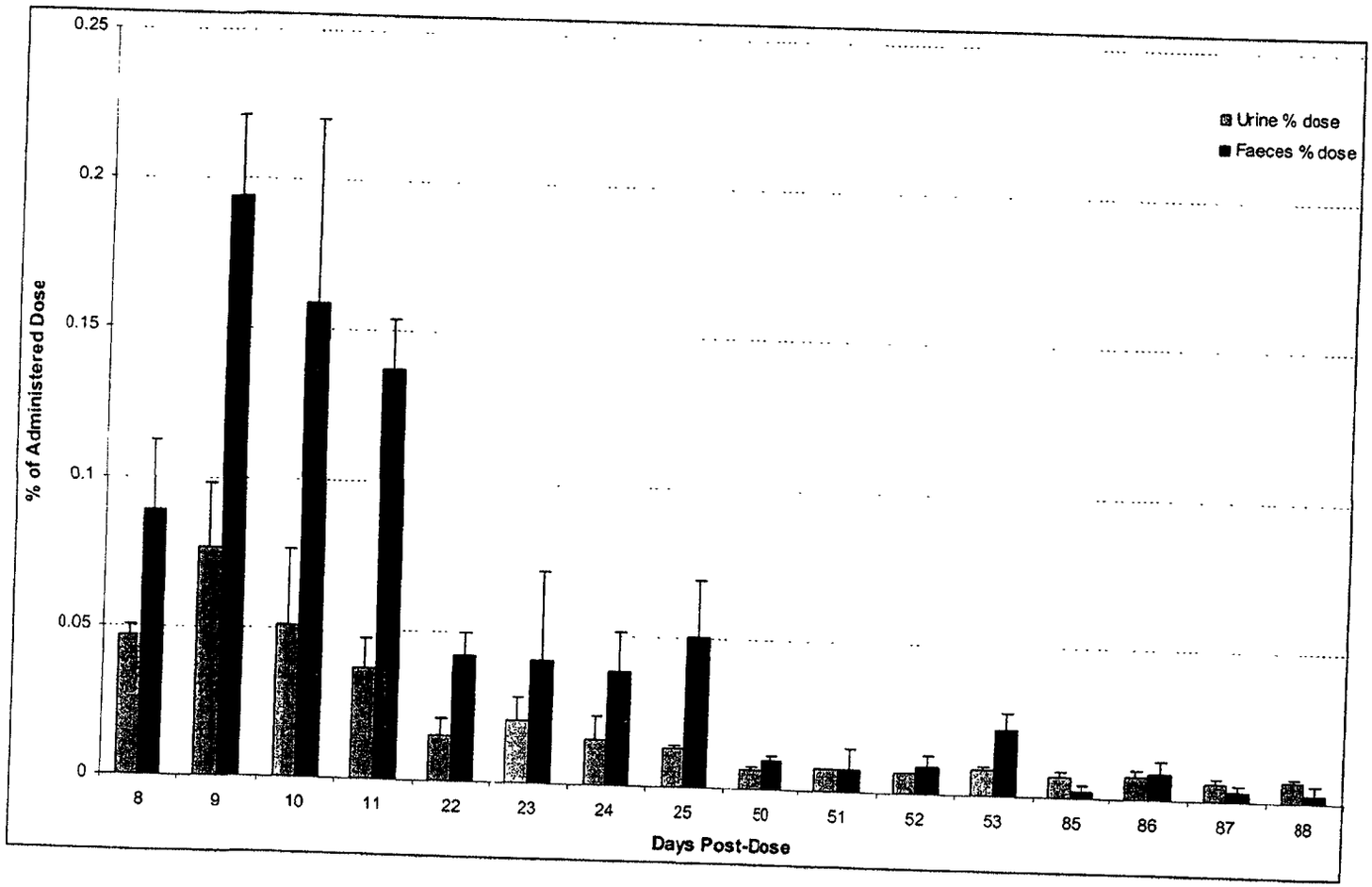


Table 7: Mean percentage of dose (n=3) recovered in urine, faeces and tissues in male rats following gavage administration of ^{14}C-MCCP at 525mg/kg

Animals	Urine	Faeces	Tissues	Total
Rats 1-3			6.895	6.895
Rats 4-6	5.943	71.439	6.258	83.640
Rats 7-9	0.211	0.579	7.864	8.654
Rats 10-12	0.065	0.172	4.008	4.245
Rats 14-15	0.029	0.049	1.377	1.455
Rats 16-18	0.028	0.017	1.672	1.717

Appendix 1. Individual Animal Data

Plasma Counts

Animal Number	DPM1	Volume (ul)	DPM per ul	Mean	SD
1	2083.90	100.00	20.84		
2	2867.10	100.00	28.67		
3	3022.70	100.00	30.23	26.58	5.03
4	479.60	100.00	4.80		
5	711.70	100.00	7.12		
6	533.10	100.00	5.33	5.75	1.22
7	254.20	100.00	2.54		
8	255.60	100.00	2.56		
9	313.20	100.00	3.13	2.74	0.34
10	110.30	100.00	1.10		
11	172.50	100.00	1.73		
12	89.80	100.00	0.90	1.24	0.43
14	67.80	100.00	0.68		
15	50.30	100.00	0.50	0.59	0.12
16	65.80	100.00	0.66		
17	68.20	100.00	0.68		
18	65.80	100.00	0.66	0.67	0.01

Blood Counts

50:50 with water

Animal Number	DPM1	Volume (ul)	Solvable	DPM per ul	Mean	SD
1	555.80	100.00	1ml	5.56		
2	535.90	100.00	1ml	5.36		
3	815.50	100.00	1ml	8.16	6.36	1.56
4	375.70	100.00	1ml	3.76		
5	376.80	100.00	1ml	3.77		
6	228.80	100.00	1ml	2.29	3.27	0.85
7	198.20	100.00	1ml	1.98		
8	202.90	100.00	1ml	2.03		
9	169.20	100.00	1ml	1.69	1.90	0.18
10	105.20	100.00	1ml	1.05		
11	134.50	100.00	1ml	1.35		
12	105.90	100.00	1ml	1.06	1.15	0.17
14	77.50	100.00	1ml	0.78		
15	97.90	100.00	1ml	0.98	0.88	0.14
16	179.60	100.00	1ml	1.80		
17	190.80	100.00	1ml	1.91		
18	186.40	100.00	1ml	1.86	1.86	0.06

APPENDIX 1 Cont.: Individual animal data

Liver Counts

3	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	64319.40	366.70	2.5ml	175.40		
2	62719.20	314.40	2.5ml	199.49		
3	165361.50	620.20	2.5ml	266.63	213.84	47.28
4	18289.90	595.40	2.5ml	30.72		
5	28623.30	612.00	2.5ml	46.77		
6	33023.70	1021.40	2.5ml	32.33	36.61	8.84
7	19041.70	1304.00	2.5ml	14.60		
8	45239.50	1482.90	5ml	30.51		
9	31329.70	1290.60	5ml	24.28	23.13	8.01
10	1064.60	204.40	2.5ml	5.21		
11	1018.30	107.20	2.5ml	9.50		
12	799.70	161.10	2.5ml	4.96	6.56	2.55
14	245.00	102.00	1ml	2.40		
15	301.20	136.00	1ml	2.21	2.31	0.13
16	537.10	114.00	1ml	4.71		
17	510.20	132.00	1ml	3.87		
18	528.40	102.00	1ml	5.18	4.59	0.67

Left Kidney Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	6204.90	129.00	1ml	48.10		
2	8225.50	148.00	1ml	55.58		
3	8421.40	137.00	1ml	61.47	55.05	6.70
4	5312.90	146.00	1ml	36.39		
5	6605.30	153.00	1ml	43.17		
6	2252.00	113.00	1ml	19.93	33.16	11.95
7	2111.80	143.00	1ml	14.77		
8	2621.20	149.00	1ml	17.59		
9	2227.10	114.00	1ml	19.54	17.30	2.40
10	739.60	137.00	1ml	5.40		
11	983.90	103.00	1ml	9.55		
12	759.80	146.00	1ml	5.20	6.72	2.46
14	699.30	118.00	1ml	5.93		
15	383.10	104.00	1ml	3.68	4.80	1.59
16	513.00	112.00	1ml	4.58		
17	1010.40	143.00	1ml	7.07		
18	634.90	114.00	1ml	5.57	5.74	1.25

APPENDIX 1 Cont.: Individual animal data

Right Kidney Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	9672.70	135.00	1ml	71.65		
2	6481.60	151.00	1ml	42.92		
3	9104.60	152.00	1ml	59.90	58.16	14.44
4	4117.10	150.00	1ml	27.45		
5	6479.00	98.00	1ml	66.11		
6	1880.40	94.00	1ml	20.00	37.85	24.75
7	1681.60	113.00	1ml	14.88		
8	2623.40	151.00	1ml	17.37		
9	2561.80	142.00	1ml	18.04	16.77	1.67
10	722.70	131.00	1ml	5.52		
11	1318.70	143.00	1ml	9.22		
12	714.00	143.00	1ml	4.99	6.58	2.31
14	600.80	144.00	1ml	4.17		
15	286.50	124.00	1ml	2.31	3.24	1.32
16	402.40	126.00	1ml	3.19		
17	442.60	128.00	1ml	3.46		
18	728.40	129.00	1ml	5.65	4.10	1.35

Spleen Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	2521.30	106.00	1ml	23.79		
2	2505.40	76.00	1ml	32.97		
3	2808.50	88.00	1ml	31.91	29.56	5.02
4	1629.20	106.00	1ml	15.37		
5	1827.60	113.00	1ml	16.17		
6	1344.70	97.00	1ml	13.86	15.14	1.17
7	1273.00	131.00	1ml	9.72		
8	897.50	96.00	1ml	9.35		
9	1167.40	93.00	1ml	12.55	10.54	1.75
10	259.10	67.00	1ml	3.87		
11	443.00	71.00	1ml	6.24		
12	297.00	69.00	1ml	4.30	4.80	1.26
14	330.70	124.00	1ml	2.67		
15	226.90	97.00	1ml	2.34	2.50	0.23
16	304.10	94.00	1ml	3.24		
17	306.60	106.00	1ml	2.89		
18	338.60	112.00	1ml	3.02	3.05	0.17

APPENDIX 1 Cont.: Individual animal data

Lungs Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	2836.00	106.00	1ml	26.75		
2	2269.60	55.00	1ml	41.27		
3	3839.90	108.00	1ml	35.55	34.52	7.31
4	1283.40	83.00	1ml	15.46		
5	1537.60	81.00	1ml	18.98		
6	2003.30	147.00	1ml	13.63	16.02	2.72
7	3190.90	142.00	1ml	22.47		
8	2189.70	106.00	1ml	20.66		
9	1754.70	105.00	1ml	16 71	19.95	2.94
10	240.20	48.00	1ml	5.00		
11	571.20	63.00	1ml	9.07		
12	466.70	117.00	1ml	3.99	6.02	2.69
14	467.20	130.00	1ml	3.59		
15	402.80	110.00	1ml	3.66	3.63	0.05
16	335.70	101.00	1ml	3.32		
17	358.20	124.00	1ml	2.89		
18	383.10	146.00	1ml	2.62	2.95	0.35

Heart Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	2007.60	113.00	1ml	17.77		
2	3095.40	142.00	1ml	21.80		
3	4057.50	142.00	1ml	28.57	22.71	5.46
4	1401.20	148.00	1ml	9.47		
5	1445.70	127.00	1ml	11.38		
6	1098.90	117.00	1ml	9.39	10.08	1.13
7	1280.20	138.00	1ml	9.28		
8	1300.90	134.00	1ml	9.71		
9	1539.80	146.00	1ml	10.55	9.84	0.65
10	445.80	126.00	1ml	3.54		
11	514.20	108.00	1ml	4.76		
12	456.40	143.00	1ml	3.19	3.83	0.82
14	177.30	101.00	1ml	1.76		
15	145.70	103.00	1ml	1.41	1.59	0.24
16	294.60	121.00	1ml	2.43		
17	295.50	125.00	1ml	2.36		
18	321.60	142.00	1ml	2.26	2.35	0.09

APPENDIX 1 Cont.: Individual animal data

Brain Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	1480.00	121.00	1ml	12.23		
2	2012.90	135.00	1ml	14.91		
3	2777.60	146.00	1ml	19.02	15.39	3.42
4	886.10	142.00	1ml	6.24		
5	898.40	119.00	1ml	7.55		
6	546.80	126.00	1ml	4.34	6.04	1.61
7	560.90	123.00	1ml	4.56		
8	609.50	129.00	1ml	4.72		
9	765.30	161.00	1ml	4.75	4.68	0.10
10	388.60	145.00	1ml	2.68		
11	390.40	104.00	1ml	3.75		
12	351.30	139.00	1ml	2.53	2.99	0.67
14	262.20	142.00	1ml	1.85		
15	278.80	104.00	1ml	2.68	2.26	0.59
16	313.10	101.00	1ml	3.10		
17	344.40	136.00	1ml	2.53		
18	351.40	121.00	1ml	2.90	2.85	0.29

Left Testicle Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	297.40	121.00	1ml	2.46		
2	2352.20	153.00	1ml	15.37		
3	1515.10	99.00	1ml	15.30	11.05	7.44
4	1071.60	154.00	1ml	6.96		
5	1184.20	127.00	1ml	9.32		
6	1508.70	133.00	1ml	11.34	9.21	2.19
7	676.00	117.00	1ml	5.78		
8	574.30	120.00	1ml	4.79		
9	563.50	100.00	1ml	5.64	5.40	0.54
10	250.40	100.00	1ml	2.50		
11	417.40	101.00	1ml	4.13		
12	273.90	128.00	1ml	2.14	2.93	1.06
14	236.30	138.00	1ml	1.71		
15	149.70	131.00	1ml	1.14	1.43	0.40
16	214.10	111.00	1ml	1.93		
17	411.40	132.00	1ml	3.12		
18	209.70	135.00	1ml	1.55	2.20	0.82

APPENDIX 1 Cont.: Individual animal data

Right Testicle Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	1879.00	144.00	1ml	13.05		
2	1858.90	126.00	1ml	14.75		
3	1520.20	100.00	1ml	15.20	14.33	1.14
4	869.50	115.00	1ml	7.56		
5	1159.40	125.00	1ml	9.28		
6	706.50	121.00	1ml	5.84	7.56	1.72
7	680.30	126.00	1ml	5.40		
8	831.00	134.00	1ml	6.20		
9	640.30	111.00	1ml	5.77	5.79	0.40
10	411.40	132.00	1ml	3.12		
11	1523.40	130.00	1ml	11.72		
12	222.60	94.00	1ml	2.37	5.73	5.20
14	174.00	121.00	1ml	1.44		
15	89.50	101.00	1ml	0.89	1.16	0.39
16	387.30	134.00	1ml	2.89		
17	212.80	150.00	1ml	1.42		
18	756.60	142.00	1ml	5.33	3.21	1.97

Fat Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	11369.80	104.00	1ml	109.33		
2	10287.50	95.00	1ml	108.29		
3	12864.70	156.00	1ml	82.47	100.03	15.22
4	17595.60	127.00	1ml	138.55		
5	15931.30	116.00	1ml	137.34		
6	12694.00	124.00	1ml	102.37	126.09	20.55
7	32529.20	145.00	1ml	224.34		
8	45203.00	149.00	1ml	303.38		
9	37296.90	112.00	1ml	333.01	286.91	56.17
10	17876.20	143.00	1ml	125.01		
11	29452.10	139.00	1ml	211.89		
12	24202.50	149.00	1ml	162.43	166.44	43.58
14	9335.50	108.00	1ml	86.44		
15	8463.50	136.00	1ml	62.23	74.34	17.12
16	9825.80	141.00	1ml	69.69		
17	7040.60	126.00	1ml	55.88		
18	7480.10	120.00	1ml	62.33	62.63	6.91

APPENDIX 1 Cont.: Individual animal data

Muscle Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	2551.70	155.00	1ml	16.46		
2	1349.60	134.00	1ml	10.07		
3	1661.10	123.00	1ml	13.50	13.35	3.20
4	1498.10	134.00	1ml	11.18		
5	799.90	120.00	1ml	6.67		
6	2125.80	155.00	1ml	13.71	10.52	3.57
7	1463.50	135.00	1ml	10.84		
8	789.60	105.00	1ml	7.52		
9	2723.40	139.00	1ml	19.59	12.65	6.24
10	441.40	113.00	1ml	3.91		
11	488.20	115.00	1ml	4.25		
12	502.60	160.00	1ml	3.14	3.76	0.57
14	216.00	144.00	1ml	1.50		
15	221.10	136.00	1ml	1.63	1.56	0.09
16	290.80	118.00	1ml	2.46		
17	214.40	98.00	1ml	2.19		
18	374.80	129.00	1ml	2.91	2.52	0.36

Skin + Fur Counts

Animal Number	DPM1	Weight (mg)	Solvable	DPM per mg	Mean	SD
1	13233.30	145.00	1ml	91.26		
2	11373.80	98.00	1ml	116.06		
3	6049.60	116.00	1ml	52.15	86.49	32.22
4	12549.70	129.00	1ml	97.28		
5	17298.10	136.00	1ml	127.19		
6	858.30	106.00	1ml	8.10	77.52	61.96
7	10128.60	98.00	1ml	103.35		
8	18108.20	146.00	1ml	124.03		
9	9771.30	103.00	1ml	94.87	107.42	15.00
10	4199.40	126.00	1ml	33.33		
11	12697.70	153.00	1ml	82.99		
12	3509.00	100.00	1ml	35.09	50.47	28.18
14	1054.50	141.00	1ml	7.48		
15	1075.10	110.00	1ml	9.77	8.63	1.62
16	1372.30	151.00	1ml	9.09		
17	2049.50	127.00	1ml	16.14		
18	870.10	105.00	1ml	8.29	11.17	4.32

Appendix 2. Urine and faeces measurements.

Note: Rat number 13 died before samples were collected

Rat No.	Sample	Urine Vol (ml)	DPM1	DPM in total urine	% dose given	Faeces Wt (g)	DPM1	DPM in total faeces	% dose given
4	24hr	6.10	48775.70	2975317.70	2.920	4.2780	692838.10	59279227.84	58.178
	48hr	5.50	16435.40	903947.00	0.887	3.6500	177757.30	12976282.90	12.735
	72hr	4.70	7419.00	348693.00	0.342	5.1142	30874.50	3157967.36	3.099
	96hr	5.00	2382.20	119110.00	0.117	11.3328	4119.90	933800.05	0.916
5	24hr	11.70	30005.50	3510643.50	3.445	2.3567	601665.70	28358911.10	27.832
	48hr	4.40	36796.90	1619063.60	1.589	5.1394	339000.40	34845173.12	34.198
	72hr	4.00	9051.40	362056.00	0.355	9.0483	18304.30	3312455.95	3.251
	96hr	3.40	4092.40	139141.60	0.137	7.6709	3560.00	546168.08	0.536
6	24hr	14.60	50597.50	7387235.00	6.921	5.5731	604813.50	67413722.34	63.155
	48hr	4.60	17618.70	810460.20	0.759	6.8737	71970.60	9894086.26	9.269
	72hr	6.90	3913.60	270038.40	0.253	6.0142	7255.80	872756.65	0.818
	96hr	4.70	2325.70	109307.90	0.102	7.3222	2404.90	352183.16	0.330
7	24hr	5.70	897.60	51163.20	0.044	4.4973	989.60	89010.56	0.076
	48hr	7.40	1261.20	93328.80	0.080	9.7506	1197.30	233487.87	0.201
	72hr	5.00	919.10	45955.00	0.039	7.1399	950.60	135743.78	0.117
	96hr	5.30	687.90	36458.70	0.031	9.7576	792.10	154579.90	0.133
8	24hr	7.00	684.30	47901.00	0.045	7.1319	868.20	123838.31	0.116
	48hr	6.40	876.80	56115.20	0.053	10.6912	818.10	174929.41	0.164
	72hr	5.80	605.50	35119.00	0.033	16.0600	760.40	244240.48	0.229
	96hr	5.00	663.10	33155.00	0.031	12.1028	541.00	130952.30	0.123
9	24hr	8.90	585.30	52091.70	0.051	4.1570	911.20	75757.17	0.074
	48hr	8.10	1209.90	98001.90	0.096	7.3055	1517.50	221721.93	0.218
	72hr	8.30	986.50	81879.50	0.080	3.3757	1990.40	134379.87	0.132
	96hr	6.10	815.00	49715.00	0.049	9.7006	816.80	158469.00	0.156
10	24hr	6.50	174.90	11368.50	0.011	9.2970	236.80	44030.59	0.041
	48hr	11.30	202.00	22826.00	0.021	1.8408	411.80	15160.83	0.014
	72hr	5.70	180.70	10299.90	0.010	11.2122	175.30	39309.97	0.037
	96hr	5.20	254.30	13223.60	0.012	8.4923	280.40	47624.82	0.045
11	24hr	6.20	383.50	23777.00	0.022	6.0664	306.40	37174.90	0.035
	48hr	4.95	614.10	30397.95	0.028	4.6495	839.30	78046.51	0.073
	72hr	4.60	565.90	26031.40	0.024	2.3089	586.60	27088.01	0.025
	96hr	2.60	592.60	15407.60	0.014	8.9200	427.40	76248.16	0.071
12	24hr	6.10	203.50	12413.50	0.012	9.9038	257.50	51004.57	0.050
	48hr	4.95	289.00	14305.50	0.014	7.4099	248.20	36782.74	0.036
	72hr	4.70	266.30	12516.10	0.012	14.2275	187.60	53381.58	0.052
	96hr	7.15	180.20	12884.30	0.013	10.8516	167.70	36396.27	0.036
13	24hr			0.00				0.00	
	48hr			0.00				0.00	
	72hr			0.00				0.00	
	96hr			0.00				0.00	
14	24hr	8.50	103.50	8797.50	0.007	4.4273	148.70	13166.79	0.011
	48hr	5.50	169.70	9333.50	0.008	2.5340	300.20	15214.14	0.013
	72hr	6.50	123.40	8021.00	0.007	2.9924	243.80	14590.94	0.012
	96hr	6.50	173.30	11264.50	0.009	8.1226	195.80	31808.10	0.026
15	24hr	7.60	75.10	5707.60	0.005	3.7665	124.90	9408.72	0.009
	48hr	6.40	128.20	8204.80	0.008	0.5639	199.70	2252.22	0.002
	72hr	3.50	212.70	7444.50	0.007	0.9425	371.50	7002.78	0.007
	96hr	6.00	138.10	8286.00	0.008	6.2568	155.80	19496.19	0.018
16	24hr	5.40	111.40	6015.60	0.005	1.4282	73.10	2088.03	0.002
	48hr	3.20	198.30	6345.60	0.006	3.4200	193.30	13221.72	0.012
	72hr	2.00	296.40	5928.00	0.005	0.1432	442.00	1265.89	0.001
	96hr	1.80	364.00	6552.00	0.006	0.1835	416.60	1528.92	0.001
17	24hr	14.10	57.00	8037.00	0.007	0.4800	77.30	742.08	0.001
	48hr	14.30	73.00	10439.00	0.009	2.4176	230.80	11159.64	0.010
	72hr	8.10	105.40	8537.40	0.008	0.6677	405.80	5419.05	0.005
	96hr	8.60	108.00	9288.00	0.008	0.1504	349.50	1051.30	0.001
18	24hr	16.40	49.00	8036.00	0.008	2.2897	91.50	4190.15	0.004
	48hr	4.80	164.60	7900.80	0.008	0.6039	307.70	3716.40	0.004
	72hr	2.40	224.20	5380.80	0.006	0.2873	666.60	3830.28	0.004
	96hr	2.30	293.00	6739.00	0.007	0.4735	639.30	6054.17	0.006

Urine, 0.1ml counted. Faeces, 0.5ml of a 10% homogenate counted

AstraZeneca

BLS3192/B

MEDIUM-CHAIN CHLORINATED PARAFFINS (C_{14-17}, 52% CHLORINATED): A comparison of acute toxicity to *Daphnia magna* using two different carrier solvents and water-accommodated fractions.

Study No: 04-0216/A

At the request of the Global Consortium of Chlorinated Paraffin Producers, the acute toxicity of two medium-chain chlorinated paraffin (MCCP) products to *Daphnia magna* was compared using three different methods to prepare the test solutions:

(i) Acetone as a solvent carrier;
(ii) Dimethylformamide (DMF) as a solvent carrier; and,
(iii) Water-accommodated fractions (WAFs).

Two test substance samples were supplied by LGC NW Limited, Runcorn, Cheshire, UK, from stocks of standard commercial material provided by manufacturers in the European Union (EU) and USA, with the following information:

Brixham Code	Source	Supplier Reference	Date sample received
04-0216	EU	JD/02	29 June 2004
04-0217	USA	RD/02	29 June 2004

Both test substances were described as medium-chain (C_{14-17}) chlorinated paraffins, chlorinated to 52% by weight, and were stored refrigerated prior to use.

The tests were carried out from 28 July to 5 August 2004. The tests on both substances using DMF and WAFs were carried out concurrently, using the same batch of test organisms. The tests on both substances using acetone were carried out concurrently during the following week.

METHODS

Acetone as carrier solvent

A stock solution (1000 mg l^{-1}) of each test substance was prepared in acetone, aliquots of which were added gradually to dilution water (Ref 1) whilst stirring by magnetic stirrer, to provide the following nominal test concentrations:

Solvent control, 0.0065, 0.013, 0.025, 0.05 and 1.0 mg l^{-1}

Equalising additions of acetone were made, giving a final acetone concentration of 0.1 ml l^{-1} in all test solutions. The solutions were stirred for a further 40 minutes before use. Volumes (200 ml) of the test solutions were transferred to duplicate glass vessels for the *Daphnia* test (see below).

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Dimethylformamide (DMF) as carrier solvent
As described above, but using DMF as the solvent in place of acetone. Stirring was for 25 minutes.

Water-accommodated fractions (WAFs)
The methods were based on ASTM Standard D6081-98 (Ref 2). The principle of the method is that a quantity of a poorly soluble substance (in excess of that which will dissolve) is stirred vigorously for a prolonged period in water, followed by separation of the aqueous phase for ecotoxicological testing.

Small amounts of the liquid test substances were placed on pre-weighed glass microscope slides, and a second glass slide used to smear the substance over the surface of the slide (sliding the inclined edge of the second slide through and across the test substance droplet, repeating if necessary with a clean slide until the appropriate weight of test substance remained on the first slide). Typically, the test substance was distributed across approximately 2 to 3 cm^2 of the slide surface. Due to the very small weights involved and the viscous nature of the test substances, it was not practical to achieve exactly the target nominal weights by this method, but the obtained weights were measured. The nominal and actual test concentrations, expressed as 'loading rates' were as follows:

Loading Rate (mg l^{-1})					
Nominal	0.1	0.32	1.0	3.2	10
Measured (EU sample)	0.144	0.356	1.17	3.19	10.5
Measured (USA sample)	0.100	0.352	1.27	3.23	10.2

For nominal loading rates below 1 mg/l, the weighed quantities were those required for 5 litres of dilution water; for the higher loading rates the weights were those required for 1 litre of dilution water.

Each slide was placed into a stainless-steel support frame and immersed within a glass vessel containing the appropriate volume (1 or 5 litres) of dilution water, equipped with magnetic stirring. The stainless steel support served to retain the slide close to the stirrer vortex whilst preventing the stirrer from colliding with the slide. The vessels were stirred for 22 hours, the stirring being sufficient to create a vortex depression of approximately 10 to 35% of the solution depth (Ref 2). After stirring, the vessels were allowed to settle for 40 minutes, after which 200 ml of each WAF was decanted to each of duplicate test vessels for the *Daphnia* test (see below). Because of the very small quantities of test substance employed in preparation of the WAFs there was no visible undissolved phase to be separated from the WAF; however, the initial 100 ml of decanted water was discarded in case there was a film of undissolved test substance on the surface. A control solution was prepared in the same manner, containing a glass slide and support frame but with no other additions.

General methods

Five *Daphnia magna* (<24 hours old) were added to each duplicate vessel of each control and test concentration (total of 10 animals per treatment) and exposed for 48 hours at 20 ± 1°C under a photoperiod of 16 h light: 8 h dark. The animals were not fed during the test and the solutions were not aerated. Immobilisation (defined as lack of whole body movement) of the *Daphnia* was recorded after 24 and 48 hours. Any flotation of the *Daphnia*

on the surface of the solutions was also recorded; after 24 hours, floating animals were re-submerged using drops of test solution from a Pasteur pipette. In the WAF treatments, several floating animals were also observed after approximately 3 hours (at each of the three highest loading rates for both substances) and were re-submerged.

The temperature of an additional vessel, containing dilution water but no animals, was measured daily by thermometer and at hourly intervals by an automatic electronic recorder. The dissolved oxygen concentration was measured at the start of the test for the control and solvent control solutions and at the end of the test for one replicate of each treatment (treatments showing 100% effect at 24 hours were measured at that time). The pH was monitored in the same manner except that excess of all solutions was measured at the start of the test. There was no analysis of the solutions for test substance concentration.

RESULTS

The percentage of the *Daphnia* affected (immobilised) in each treatment after 24 and 48 hours is shown in Table 1 (Acetone and DMF) and Table 2 (WAFs). The percentage of the *Daphnia* floating on the surface of the solutions is also given.

There was zero effect (and zero flotation) in all the control and solvent control solutions. It was noted (for all methods and both substances) that in treatments showing an immobilisation effect, ≥80% of the affected animals would also have been recorded as dead (no visible signs of life when viewed by eye) if that criterion had been applied.

As has been observed in previous studies with MCCP (eg Ref 3), the effect data after 24 hours generally showed an erratic concentration-response relationship. This was probably because the effects on the test organisms were just starting to be exerted after that period of time, resulting in a rapidly changing percentage effect pattern. Therefore, it is doubtful whether any valid comparisons can be made between test methods or test substances based on the 24-hour data.

After 48 hours, there was ≥90% immobilisation of the *Daphnia* at all concentrations of both test substances when using both acetone and DMF as carrier solvent. Because 'no effect' levels were not obtained, it is not possible to conclude that the EC50 values would be the same for both these solvents. However, it is reasonable to conclude that there was no substantial reduction in acute toxicity when using DMF, compared with acetone, which was one of the hypotheses being tested by this experimental design. Similarly, although it was not possible to compare the EC50 values, there was no evidence of any substantial difference in acute toxicity between the EU and USA samples, when using these two carrier solvents. The results obtained with acetone are in reasonable agreement with the data obtained previously using acetone for a different sample of MCCP of European origin (Ref 3). In that previous study, 45% effect was obtained at a nominal concentration of 0.0056 mg l^{-1}, compared with 100 and 90% effect (EU and USA samples, respectively) at a nominal 0.0065 mg l^{-1} in the present study.

When tested using WAFs, both samples gave >50% effect after 48 hours at (and above) a nominal loading rate of 0.32 mg l^{-1}. The USA sample showed a somewhat lower toxicity than the EU sample; however, further work would be necessary to confirm this was a real difference. There was considerable flotation of the *Daphnia* in a number of the WAF treatments, which indicates the presence (and physical effect) of undissolved test substance in the test solutions. It should be noted from Table 2 that the flotation percentages include both

affected and unaffected animals and, because floating animals were re-submerged after 3 and 24 hours, the data should only be considered as semi-quantitative. Flotation of *Daphnia* is often observed with such very hydrophobic test substances. Although flotation does not necessarily lead to the immobilisation of the organism, the extent of its possible contribution to the percentage effect cannot be determined reliably and may vary between different test substances.

Comparing the solvent carrier results with those derived using WAFs, it can be concluded that toxicity of MCCP to *Daphnia* is not restricted to tests involving organic carrier solvents. However, it should be noted that the lowest WAF loading rate (0.1 mg l^{-1}) was more than an order of magnitude higher, but gave an apparently lower effect (at least for the USA sample), than the lowest concentration tested using solvents (0.0065 mg l^{-1}). The WAF loading of 0.1 mg l^{-1} was also approximately an order of magnitude higher than the solubility limit for MCCP (0.005 to 0.027 mg l^{-1}). With such an excess of material, it would be expected that the MCCP would have dissolved up to the solubility level, and therefore had the same effect, at all WAF loading rates.

Temperature during the test was within the nominal range of 20 ± 1°C. The dissolved oxygen and pH of the solutions ranged from 8.8 to 9.4 mg l^{-1} and 7.8 to 8.1, respectively, which were considered normal and satisfactory.

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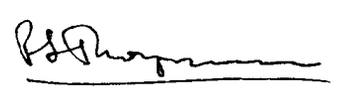

13 Sept 2004.

R S Thompson
Study Director

TABLE 1

MCCP: Acute toxicity to *Daphnia magna* of EU and USA samples using acetone and DMF as carrier solvents

Nominal concentration ($mg\ l^{-1}$)	Exposure time	% *Daphnia* affected (% Flotation*)			
		EU sample (04-0216)		USA sample (04-217)	
		Acetone	DMF	Acetone	DMF
Solvent control	24 hours	0	0	†	†
0.0065		20	0	30	60
0.013		30	70	50	90
0.025		30	100	30	70
0.05		20	90	60	90
0.1		30	70	20	100
Solvent control	48 hours	0	0	†	†
0.0065		100 (20)	100	90	100
0.013		100	100	100	100
0.025		100	100	100	100
0.05		100	100	100	100
0.1		100	100	100	100 (40)

* Flotation indicated if >10%

† Shared solvent control for EU and USA samples

TABLE 2

MCCP: Acute toxicity to *Daphnia magna* of EU and USA samples tested using water-accommodated fractions (WAFs)

WAF Loading rate ($mg\ l^{-1}$)			Exposure time	% *Daphnia* affected (% Flotation*)	
Nominal	Measured #			EU sample (04-0216)	USA sample (04-0217)
	EU sample	USA sample			
Control	-	-	24 hours	0	†
0.1	0.144	0.100		20	0
0.32	0.356	0.352		0 (20)	0
1.0	1.17	1.27		40 (60)	0 (90)
3.2	3.19	3.23		90 (30)	50 (80)
10	10.5	10.2		70 (60)	60 (40)
Control	-	-	48 hours	0	†
0.1	0.144	0.100		90 (20)	20 (60)
0.32	0.356	0.352		90 (60)	60 (50)
1.0	1.17	1.27		100	70 (70)
3.2	3.19	3.23		100	100
10	10.5	10.2		100 (20)	100

* Flotation indicated if >10%

† Shared control for EU and USA samples

\# Measured loading rates based on weighed quantity of test substance added to dilution water

AstraZeneca

BLS3308/B

LONG-CHAIN CHLORINATED PARAFFIN ($C_{>20}$, 43% CHLORINATED): Determination of acute toxicity to *Daphnia magna*.

Study No: 05-0365/A

At the request of the Global Consortium of Chlorinated Paraffin Producers, the acute toxicity of Long-Chain Chlorinated Paraffin ($C_{>20}$, average 43% chlorinated) (LCCP) to *Daphnia magna* was determined. The test substance sample was supplied by Dover Chemical Corporation, Dover, Ohio 44622, USA, on 2 November 2005 under the trade name Paroil 140 (unstabilised) with the batch reference number 23A1028-1. The carbon chainlength distribution of the raw material used to produce the test substance showed that >96% had a chainlength of $C_{>20}$ with a peak at C_{25} (Fig 1). The average chainlength by weight was C_{26}. The sample was stored frozen prior to use. The test was carried out from 16 to 18 November 2005, according to OECD Guideline 202 (Ref 1).

METHODS

The test solutions were prepared as Water-Accommodated Fractions (WAFs) using a method based on ASTM Standard D6081-98 (Ref 2). The principle of the method is that a quantity of a poorly soluble substance (in excess of that which will dissolve) is stirred vigorously for a prolonged period in water, and the aqueous phase is taken for ecotoxicological testing.

Small amounts of the liquid test substance were placed on pre-weighed glass microscope slides, and a second glass slide used to smear the substance over the surface of the slide (sliding the inclined edge of the second slide through and across the test substance droplet, until the appropriate weight of test substance remained on the first slide). Typically, the test substance was distributed across approximately 1 to 3 cm^2 of the slide surface, depending on substance weight. The weighed quantities were those required for 1.8 litres of dilution water. The nominal and actual test concentrations, expressed as WAF 'loading rates' were:

Nominal loading rate ($mg\ l^{-1}$)	Nominal weight (mg)	Measured weight (mg)	Actual loading rate ($mg\ l^{-1}$)
0.20	0.36	0.37	0.21
1.0	1.80	1.83	1.0
5.0	9.00	9.19	5.1

Each slide was placed into a stainless-steel support frame and immersed within a glass vessel containing 1.8 litres of dilution water (Ref 3), equipped with magnetic stirring. The stainless steel support served to retain the slide close to the stirrer vortex whilst preventing the stirrer from colliding with the slide. The vessels were stirred for 24 hours, the stirring being sufficient to create a vortex depression of approximately 35% of the solution depth. After stirring, the vessels were allowed to settle for 50 minutes, although there was no visible undissolved phase due to the small test substance quantities. After settlement, 200 ml aliquots of each WAF was drawn off from below the surface, via a side-arm in the vessel, and

transferred to each of 4 replicate *Daphnia* test vessels (see below). The initial 200 ml of water was discarded to flush the line. A control solution was prepared in the same manner, containing a glass slide and support frame but with no other additions.

Five *Daphnia magna* (<24 hours old) were added to each replicate vessel (total of 20 animals per treatment) and exposed for 48 hours at 20 ± 1°C under a photoperiod of 16 h light: 8 h dark. The animals were not fed during the test and the solutions were not aerated. Immobilisation (defined as lack of whole body movement) of the *Daphnia* was recorded after 24 and 48 hours. Any flotation of the *Daphnia* on the surface of the solutions was recorded. After 24 hours, any floating animals were re-submerged.

The temperature of an additional vessel, containing dilution water but no animals, was measured daily by thermometer and at hourly intervals by an automatic electronic recorder. The pH and dissolved oxygen concentration of the control and highest test substance concentration were measured at the start (excess test solution) and finish of the test (two replicates). There was no analysis of the solutions for test substance concentration.

RESULTS

The percentage of the *Daphnia* affected (immobilised) in each treatment after 24 and 48 hours is shown in Table 1. The percentage of the *Daphnia* floating on the surface of the solutions is also given.

There was zero effect in the control and all test substance concentrations after 48 hours. Slight flotation (10 to 15%) was observed at nominal loading rates of 0.2 and 1.0 mg l^{-1} after 24 hours, but following re-submergence of these animals no further flotation was observed.

Temperature during the test was within the nominal range of 20 ± 1°C. The dissolved oxygen and pH of the solutions ranged from 9.0 to 9.2 mg l^{-1} and 8.11 to 8.14, respectively, which were considered normal and satisfactory.

DISCUSSION AND CONCLUSIONS

The 48h EC50 to Daphnia magna was >5.1 mg l^{-1} (WAF loading rate). In a previous study (Ref 4), the same WAF methodology had been shown to reveal the toxicity of shorter chainlength chlorinated paraffins at loading rates below 1 mg l^{-1}.

The water solubility of a similar 43%-chlorinated LCCP has been determined to be 0.0064 mg l^{-1} by radiochemical analysis and <0.005 mg l^{-1} by specific analysis (Ref 5). Therefore, all the loading rates used in the present study would be expected to result in a saturated solution of the test substance. It can be concluded that the test substance is not acutely toxic to *Daphnia* at the water solubility limit.

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30 November 2005

R S Thompson
Study Director

TABLE 1

LONG-CHAIN CHLORINATED PARAFFIN ($C_{>20}$, 43% chlorinated): Acute toxicity to *Daphnia magna*

WAF loading rate (mg l^{-1})		% *Daphnia* affected (% Flotation)	
Nominal	Actual	24 hours	48 hours
Control	-	0	0
0.2	0.21	0 (10)	0
1.0	1.0	0 (15)	0
5.0	5.1	0	0

FIGURE 1

CARBON CHAINLENGTH DISTRIBUTION OF PARAFFIN RAW MATERIAL